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CYTOLOGIC AND GENETIC STUDIES OF VARIABILITY OF STRAINS OF WHEAT DERIVED FROM INTERSPECIFIC CROSSES [1]

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INTRODUCTION

The possibilities of combining the desirable characters of the *vulgare* and emmer groups of wheat in a single variety have received considerable attention from plant breeders. Special emphasis has been given to the breeding of a wheat that would combine the resistance to black stem rust (*Puccinia graminis tritici* Erikss, and Henn.) of the emmer group with the milling and baking qualities and other desirable characters of the *vulgare* group. At the Minnesota Agricultural Experiment Station a cross of Marquis with a highly rust-resistant variety of durum called Iumillo was made in 1914, from which Marquillo was produced. This variety possessed 42 chromosomes, high-yielding ability, resistance to stem rust, and stiff straw. When subjected to milling and baking tests, Marquillo wheat appeared to yield a flour which could be converted into bread that was satisfactory in every particular except perhaps in the matter of color.

Marquillo was a distinct step in advance, but there was still room for improvement. Accordingly, homozygous rust-resistant lines from the cross of Marquis × Iumillo were crossed with Kanred × Marquis selections, which excelled in agronomic characters. Minnesota 2303 is a selection from this cross which gives considerable promise, being rust resistant, possessing desirable agronomic characters, and apparently milling and baking qualities equal to those of Marquis.

The investigators at the University of Minnesota realized that Marquillo exhibited somewhat greater variability in agronomic characters than such varieties as Marquis. Cytological research was started in an endeavor to determine the germinal stability of this variety. As the work progressed it seemed desirable to add Marquis to the studies for purposes of comparison and Minnesota 2303 was included in a part of the study because it has considerable promise of becoming a highly desirable economic variety. The results are reported in this paper.

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REVIEW OF LITERATURE

The literature on the cytology of Triticum, especially as it pertains to phylogeny, has been reviewed by Aase (*1*).[3] Watkins (*18*) gives a critical account of the present knowledge of the origin and genetic relationship of the wheat species, and their cytological and genetic behavior when crossed. For a more extensive review of literature than is given here the reader is referred to their articles. In this paper only that part of the literature is considered which has a direct bearing on the problem under discussion.

Huskins (*10*) states that his colleague, J. Philp, has found chromosome irregularities in the F_1 plants resulting from crosses of hexaploid oats. Huskins (*10*) also found irregular chromosome behavior in a cross between *Triticum vulgare* and *T. spelta* made by Nilsson-Leissner. Huskins states that Nilsson-Leissner found this cross to segregate commonly for type of glume in a 3:1 ratio; but sometimes in almost the reverse proportions, and a number of abnormal types appeared.

Sapehin (*15*), working with pure lines of wheat and F_1 crosses between 42-chromosome wheats, distinguished two types of anomalies which occur during meiosis. The first of these types is characterized by the occurence of univalents; the second type by a disorderly arrangement of the chromosomes which readily vacuolize. Sapehin states that by cytological investigation he has proved that spore formation does not proceed normally in all pure lines, but that there occur anomalies of the first type in numbers from a fraction of 1 per cent of the cells to several per cent. He makes the following statement (*15, p. 164*):

> Of special interest in this regard is the best yielder among the author's pure lines of soft spring wheat, *Tr. milturum* 00180, showing every year up to 20–30%, and sometimes even up to 50–60%, of spore mother cells of anomalies of the second type. * * * as the author's investigations have shown, spore formation in F_1 00180×other 42-chromosome wheats displays on the whole normal pictures, no matter whether 00180 has been the mother or the father plant.

Sapehin found that many anomalies were produced in crosses of Ukrainian lines with "related" or "identical" varieties from Afghanistan and eastern Siberia.

On the basis of ratios in which the heterozygous speltoids segregate, Nilsson-Ehle (*13*) divided speltoids into three types, A, B, and C. In type A the ratio approximates 1:2:1 (564 normal segregates:757 heterozygous speltoids:165 homozygous speltoids), but the homozygous speltoids were deficient. He found that heterozygotes of type B gave very few homozygous speltoids, and the heterozygotes were more numerous than would be expected on a 1:2:1 basis. A total ratio of 317 normal segregates:1,300 heterozygous speltoids:13 homozygous speltoids was obtained. His heterozygotes of type C again gave very few homozygous speltoids but gave more normals than heterozygotes, a total of 491 normal segregates:456 heterozygous speltoids:8 homozygous speltoids. However, this difference between the normals and heterozygotes is not great. Nillson-Ehle found that in most cases the three types A, B, and C are easily distinguished by their different ratios; but occasionally a ratio may leave the matter doubtful, for example, 120:188:1.

[3] Reference is made by number (italic) to Literature Cited, p. 831.

Huskins (*9*) found that in speltoids of type A furnished him by Akerman the gametes all had 21 chromosomes. No deviation from the normal arrangement of 21 bivalents was found in the normal plants. The heterozygous speltoid plant showed a trivalent and a univalent in many of its pollen mother cells, though naturally it was not always possible to prove the existence of both in the same cell. The homozygous speltoid plant showed a quadrivalent in many of its pollen mother cells and a trivalent was seen in two of them.

Huskins (*9*) examined speltoids of the B type furnished by Nilsson-Ehle. The normal plants have the normal chromosome number and normal divisions. Each of the heterozygotes has only 41 chromosomes. In almost every case these formed 20 bivalents and 1 univalent. According to Huskins, Akerman has described a strain that differs from the ordinary B type strains in having produced some moderately vigorous and fertile homozygous speltoid progeny instead of only sterile dwarf ones. Huskins (*9*), from 30 seeds of a descendant homozygous speltoid plant sown in November, 1926, obtained 10 homozygous speltoid progeny. One of these was examined cytologically and found to have only 41 chromosomes. The behavior of this one was different, however, from that found in the 41-chromosome heterozygote of preceding strains. The odd chromosome was seen dividing on the plate during the anaphase in only about 75 per cent of the cells examined. A trivalent instead of a univalent was seen in a very large number of cells. These trivalents were of various shapes. In no case was a trivalent found to be accompanied by one univalent in this plant. Split univalents going at random to either pole were seen in many second divisions.

Huskins (*9*) found the heterozygous speltoid plants of type C to have 43 chromosomes, the homozygous speltoids were all rather weak, more or less sterile, and had 44 chromosomes. At the first metaphase of the heterozygous speltoids the chromosomes were believed to be arranged usually as 20 pairs and 1 trivalent. The method of pairing of the trivalent was usually end to end. All the 44 chromosome C types were very irregular cytologically.

Huskins (*9*), in studying the speltoids, noted further abnormalities. In one case the loss of approximately half a chromosome was noted and in another two of the members of a trivalent showed distinct subterminal constrictions. One of Huskins' illustrations of the metaphase of the first division shows a pair off the equatorial plate.

Huskins (*8*) reports the occasional occurrence of laggard and vagabond chromosomes in varieties of *Avena sativa*. He also reports that all the type 1 fatuoids had 42 chromosomes. In the normal segregates cytological conditions were found to be more irregular than in normal pure-line varieties of *A. sativa*. The presence of laggard and vagabond chromosomes was noted more frequently, and fewer cells showed perfectly regular splitting of the bivalents in the first anaphase.

MATERIALS AND METHODS

In 1929 seed of Marquillo was space planted in 5-foot rows so as to make possible individual plant studies. Two spikes on each of the resulting plants were bagged and both bagged and nonbagged spikes were harvested the following fall. Later the percentage of fruitfulness was determined on both the bagged and nonbagged heads. Cytologic

material was taken from plants selected at random and the entire florets of spikelets were killed and fixed in Allen's modification of Bouin's solution. All cytologic studies were made from permanent paraffin sections stained by using Newton's iodine-gentian-violet method as described by Huskins (*8*).

Sections were cut 15μ thick and lengthwise of the spikelet, with the exception of a very little of the earlier prepared material, which was cut transversely. This method of handling the material provided a side view of a majority of the achromatic figures of the microsporocyte, greatly facilitated the studies, and enhanced the ease with which they could be made, as a greater number of microsporocytes or microspores could be studied in one section. Sufficient end views of achromatic figures were obtained for purposes of comparison and the making of chromosome counts. The use of entire spikelets was also particularly advantageous as sections were frequently found which showed a number of the stages of sporogenesis. For example, a single section has been found showing the prophase of the first division, diakinesis, metaphase of first division, diads, and nearly mature microspores.

All counts of chromosomes were made from a side or end view of the metaphase or early anaphase of the first meiotic division of microsporogenesis. The number of chromosomes was determined by making counts from those cells having the chromosomes distinctly separated, and, therefore, easily distinguishable. (Pl. 4, A, D, and F.) In most plants it was possible to get good counts from both side and end views of the mataphase as well as the anaphase. Determination of chromosome number from side views was made by the aid of camera-lucida drawings. (Pl. 4, C and E.)

The studies of the occurrence of micronuclei were made on immature microspores still grouped together in tetrads in practically all cases, but in a few of the plants the microspores had broken apart and no longer exhibited this arrangement. However, in these latter plants the nucleus stained well and the microspores did not present the wrinkled condition characteristic of mature pollen grains. Microspores through which the knife had passed were not included in the counts, and only micronuclei well embedded in the cytoplasm were counted.

Of the material grown in 1929, 30 plants were studied both cytologically and genetically. The progeny of three of these plants were selected for further cytologic and genetic studies the next year, for the following reasons. Plant 407–12–3 had only 41 chromosomes, plant 407–17–13 had the normal number of chromosomes but showed considerable irregularity in chromosome behavior during microsporogenesis, and plant 407–12–24 possessed 42 chromosomes and was found to have considerable regularity of chromosome behavior during microsporogenesis. Marquis was included in these studies for purposes of comparison. All seeds were sprouted in germinators, transplanted into pots in the greenhouse, and later the resulting plants were transplanted into the field, being placed in 5-root rows, 1 foot apart and the plants spaced 6 inches within the row. Correlated data were kept on cytologic phenomena, germination, vigor of plants, survival, number of spikes per plant, height of individual plants, fruitfulness, weight of seed per plant, and certain qualitative characters. The material from which cytologic studies on Minnesota 2303

were made, was obtained from plants spaced 3 inches apart in 5-foot rows.

The progeny of 23 of the 30 plants studied cytologically were grown in 1930 for the purpose of correlating morphological and size variations under field conditions with previously determined cytologic irregularities and also for the purpose of determining the amount of natural crossing. Only seed from spikes that had been bagged the previous year was used in planting. The plantings were made in 5-foot rows 4 inches apart and the seed was spaced 4 inches within the row. First, two rows of Ceres were sown, then two days later a row of Marquillo, and two more rows of Ceres. This method of planting provided for two rows of Ceres on one side of Marquillo planted two days earlier and two rows on the other side planted on the same date as Marquillo. During the summer two heads of each plant of Marquillo were covered before pollination had occurred and the remaining spikes were left uncovered. Notes were taken on emergence, survival, number of spikes per plant, height of individual plants, fruitfulness, weight of seed per plant, and certain qualitative characters.

Only the outside florets of the noncovered seed were used in determining percentage of fruitfulness. Coefficients of variability were calculated for the percentage of fruitfulness in outside florets of noncovered spikes, center florets of noncovered spikes, outside florets of covered spikes, and center florets of covered spikes. The coefficients of variability were 16.5 ± 1.30, 117.2 ± 6.16, 22.3 ± 1.78, and 131.9 ± 12.96, respectively. These results show that the center florets in noncovered and covered spikes varied greatly in percentage of fruitfulness and that the outside florets of the bagged spikes varied more than nonbagged spikes. The average percentage of fruitfulness in the outer florets of the noncovered spikes was 85.1 ± 0.92 as compared to 18.9 ± 2.09 for center florets, 78.8 ± 1.33 for outer florets of covered spikes, and 12.9 ± 2.10 for center florets of covered spikes. Because of these results only the outside florets of noncovered spikes were used as a measure of fruitfulness.

In tests conducted in the greenhouse during the winter of 1929–30, progeny of eight plants of Marquillo in the seedling stage showed pronounced resistance to *Puccinia graminis tritici*, physiologic form 21. Ceres tested at the same time showed susceptibility. To determine the amount of natural crossing in these lines, seeds from bagged spikes of these plants were planted in the manner outlined above. During the summer two heads of each plant were bagged and those remaining were left uncovered. The following winter progeny from both covered and noncovered spikes were tested in the greenhouse for resistance to black stem rust, form 21. Since susceptibility is dominant in a cross between Marquillo and Ceres the plants resulting from cross fertilization were readily distinguishable.

TERMINOLOGY

Certain terms used in discussing the different chromosomal aberrations found occurring during microsporogenesis may need some explanation. Nonorientation of bivalents has been applied to the occurrence of a bivalent or bivalents off the equatorial plane just prior to disjunction of the main group of bivalents during metaphase

of sporogenesis. (Pl. 1, C and D.) Nonconjunction is used to signify the occurrence of a univalent or univalent chromosomes during the metaphase of the reductional division, when presumably the homologous mate or mates of this univalent or these univalent chromosomes are present. (Pl. 3, D.) Polyvalence has been employed to designate the union of three or more chromosomes during the metaphase of the reductional division. (Pls. 2, A, and 3, F.) Predisjunction is used to denote the disjunction of a bivalent or bivalents in advance of the main group of conjugated chromosomes during metaphase of the reductional division. (Pl. 3, E.)

CYTOLOGIC STUDIES OF ABERRATIONS

CHROMOSOME NUMBERS AND THE BEHAVIOR OF UNIVALENTS IN 41-CHROMOSOME PLANTS

The number of chromosomes was determined for 32 plants of Marquillo grown in 1929, and 27 plants of Marquis grown in 1930. The plants of both Marquillo and Marquis were unselected, and may be considered as representative in so far as samples composed of such small numbers can be representative. All the plants of Marquis and all but 2 of the 32 plants of Marquillo were found to have the normal somatic chromosome number of 42. The somatic chromosome number of the other 2 plants of Marquillo, culture numbers 407–15–17 and 407–12–3, was 41.

A study of the different stages of microsporogenesis of these 41-chromosome plants is of interest not only because it furnishes further evidence as to the authenticity of the counts, but also because it supplies information as to the behavior of the univalent chromosome during sporogenesis. This information is valuable in an analysis and an interpretation of the significance of the chromosomal aberrations which are discussed later.

In plant 407–15–17 microsporocytes showing different stages were available—diakinesis, metaphase, late anaphase of the second division, telophase of the second division, and young microspores not having undergone complete separation. (Pl. 5, A–F.) It was impossible to identify the univalent chromosome in the diakinesis stage, as can be seen from an examination of Plate 5, A. All but three of the chromosomes in this figure showed their paired nature, and any of these three may well have been the univalent. The univalent chromosome was clearly distinguishable in 84 of the 101 pollen mother cells examined during the metaphase of the first division. Plate 5, B, shows 20 pairs lined up on the equatorial plane and 1 single and a fragment not on the equatorial plane. Plate 4, F, is an end view of the metaphase of the same plant, showing 20 bivalents and 1 univalent chromosome. Diads of the first division and metaphases of the second division were not available, but the sections showed some microsporocytes in the anaphase of the second division, and lagging chromosomes were present. (Pl. 5, C.) Probably only 2 of the 4 chromosomes not at the poles are due to the univalent chromosome of the metaphase. Plate 5, D–F, shows the behavior of the lagging chromosomes during reconstruction of the nuclei after the second division. In the left half of Plate 5, E, is shown a chromosome which has been divided transversely, presumably because of the formation of the cell plate. This is believed to be of very little sig-

nificance in relation to the fragmentation reported later, as it is extremely doubtful whether these parts of chromosomes would be passed on to the next generation. The behavior of the lagging chromosomes during the metaphase of the first division, anaphase of the second division, and telophase of the second division strongly indicates that these small clumps of chromatin are the result of lagging chromosomes which have formed micronuclei, and they will be considered as such in the remaining part of this paper. Complementary micronuclei are of common occurrence in young microspores of interspecific wheat hybrids which are semihaploid. (Kihara (*11*), Watkins (*17*), Aase (*1*).)

Of 185 immature microspores examined, 30.3 per cent showed micronuclei, which, as will be shown later, is strong evidence that the microsporocytes had one univalent chromosome during sporogenesis. Very infrequently cases were found in which a cell wall had been laid down between the macronucleus and the micronucleus, thus forming miniature microspores.

Plant 407–12–3 was the other one of the 32 examined that had 41 somatic chromosomes, as determined by counts made from both the anaphase of the first division and side view of the metaphase. Plate 4, C, shows drawings of 20 bivalents and 1 single made from the side view of the metaphase of the first division. The counts on chromosome number are further substantiated by the fact that of the 63 pollen mother cells studied in the metaphase stage 57, or 90.5 per cent, showed the single chromosome. Moreover, it was possible to find lagging chromosomes in the late telophase of the second division that gave indications of forming micronuclei. Of the 667 immature microspores observed in the tetrad stage, 172 possessed both a macronucleus and a micronucleus. This is 25.8 per cent of the number examined. Of this 25.8 per cent, the majority had only 1 micronucleus, but infrequently 2, 3, or 4 were present, the latter being the highest number found in 1 microspore. More than 1 micronucleus in a single microspore is probably due to some of the chromosomal aberrations discussed later.

The occurrence of plants within a variety having one less than the normal chromosome number raises the question as to their origin. It will be remembered that Marquillo is descended from a cross between Marquis (42 somatic chromosomes) and Iumillo (28 somatic chromosomes). Are the 41-chromosome plants occurring in the fifteenth generation after the cross descendants of plants which have not attained the chromosome number normal to *Triticum vulgare* or are they descendants of 42-chromosome plants and owe their origin to irregular behavior of chromosomes during meiosis? The chromosomal aberrations which are described below may be of some aid in answering this question.

OCCURRENCE OF MICRONUCLEI IN 42-CHROMOSOME PLANTS

Thirty plants of Marquillo and Minnesota 2303 and 27 plants of Marquis were studied for the occurrence of micronuclei. The data given in Table 1 show that 2.8 ± 0.16 of the microspores of Marquillo showed this condition, and 0.8 ± 0.04 of those of Minnesota 2303, and 0.8 ± 0.06 of those of Marquis. One plant of Marquillo had as high as 7.6 per cent of the microspores showing micronuclei and another as low as 1.1 per cent, whereas the range in Minnesota 2303 was from 0.3

per cent to 1.7 per cent and that of Marquis from 0 to 2.2 per cent. There was only one plant of Marquis which failed to show the occurrence of micronuclei. As can be readily seen from an examination of the probale errors of the means, the difference noted between Marquis and Marquillo is statistically significant, and the means for Marquis and Minnesota 2303 are the same. In regard to the percentage occurrence of micronuclei, Marquis had a coefficient of variability of 53.7, Marquillo 46.0, and Minnesota 2303, 38.7. The data for Marquillo were based upon an examination of 18,644 microspores, those for Marquis upon 15,844 microspores, and those for Minnesota 2303 upon 20,944 microspores, making an average of somewhat over 500 microspores per plant. Only a few plants were included from which less than 500 microspores were studied; the lowest number was 382.

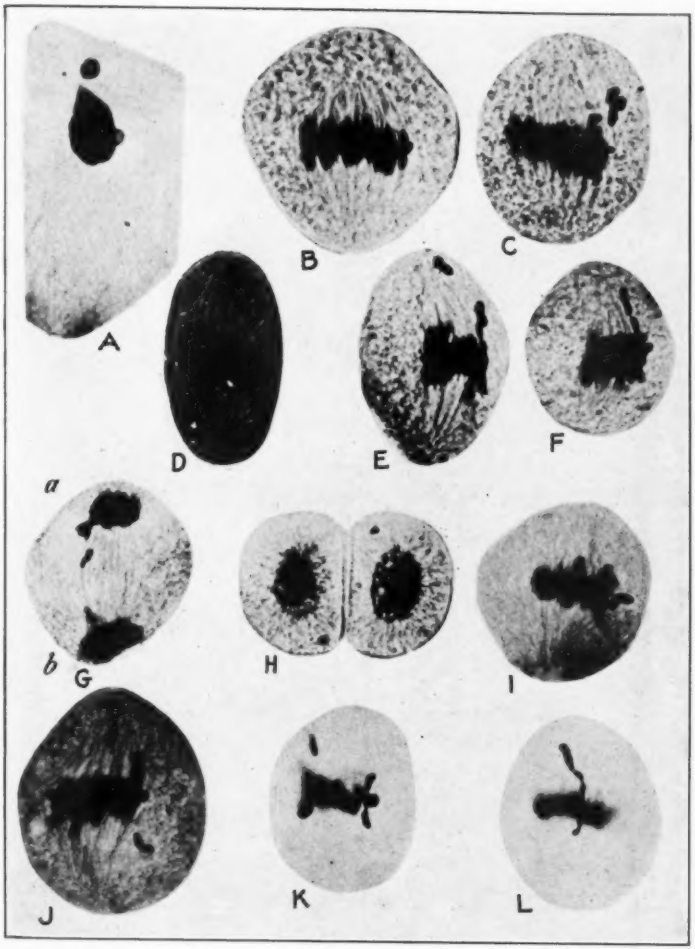
TABLE 1.—*Average percentage and coefficient of variability of microspores showing micronuclei*

Variety or culture	Number of plants	Microspores		
		Total	Micronuclei	
			Average	Coefficient of variability
Marquillo	30	18,644	2.8±0.16	46.0
Marquis	27	15,844	.8± .06	53.7
Minnesota 2303	30	20,944	.8± .04	38.7

It should be noted that 30.3 per cent of the microspores showed micronuclei in plant 407–15–17 and that 25.8 per cent revealed this phenomenon in plant 407–12–3. Micronuclei were present in 2.8 per cent of the microspores of the other 30 plants of Marquillo and in 0.8 per cent of the microspores of Minnesota 2303 and Marquis. Plate 1, H, is a photomicrograph of microspores of Marquillo showing micronuclei. It has been shown that the micronuclei in the 41-chromosome plant probably are formed from the univalent chromosome. The origin of the micronuclei in the 42-chromosome plants remains to be determined.

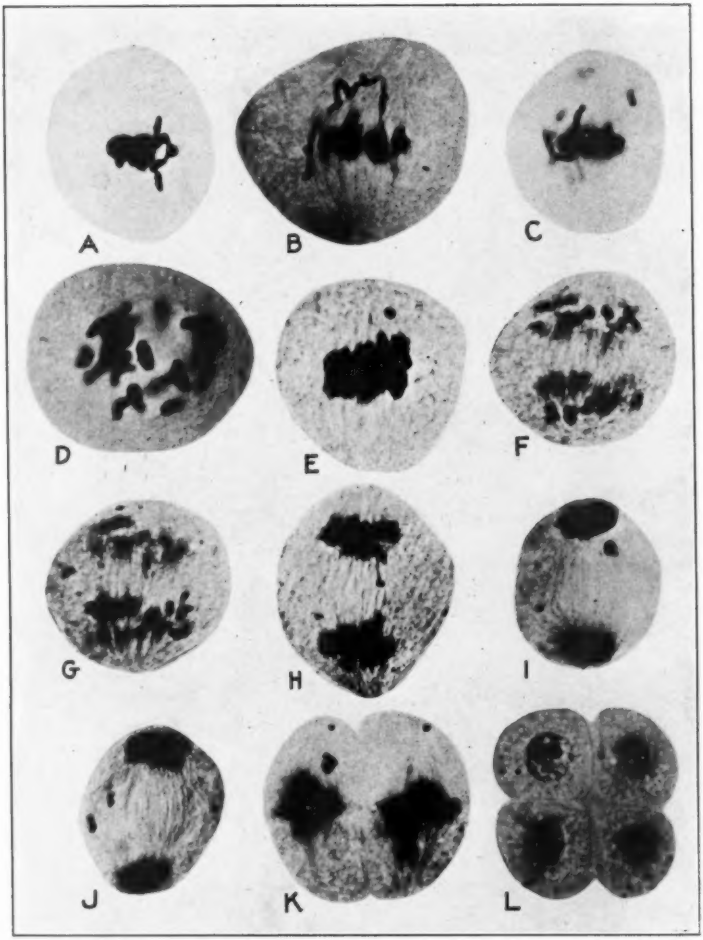
EXTRUSION OF KARYOTIN

Plate 1, A, is a photomicrograph of a very young microsporocyte of Marquillo culture, 407–15–17 (41 somatic chromosomes), in which karyotin is being extruded from the nucleus. Both the nucleus and extruded karyotin were deeply embedded in the cytoplasm and, therefore, could not have been touched by the microtome knife. At first it was thought that the extruded karyotin might be the nucleous, but as can be seen from the photomicrograph, which also shows the nucleolus being extruded into the nuclear cavity, the two differ materially in structure and staining reaction. Of the cells studied for this phenomenon, some in later stages showed a nucleolus in the nuclear cavity, but no cells were found in which the nucleolus was in the surrounding cytoplasm. The amounts of the karyotin varied from those as large as that shown in Plate 1 to those approximately one-third of that size. Plant 407–15–17 showed extrusion of larger



Stages in the meiotic divisions, microsporocytes; photomicrographs (× 1,500)

A.—Extrusion of karyotin; early liptotene stage; Marquillo.
B.—Regular equatorial plane; metaphase of first division; Marquillo.
C.—Nonorientation of a bivalent; metaphase of first division; Marquillo.
D.—Nonorientation of a bivalent; metaphase of first division; Marquis.
E.—Nonconjunction or predisjunction and nonorientation; metaphase of first division; Marquis.
F.—Predisjunction and nonorientation; metaphase of first division; Marquis.
G.—Lagging chromosomes *a*, reductional division only; *b*, reduction division and equational division; telophase of first division; Marquis.
H.—Micronuclei; microspores arranged in tetrads; Marquillo.
I.—Univalent on the equatorial plane; metaphase of first division; 41-chromosome plant of Marquillo.
J.—Univalent off the equatorial plane; metaphase of first division; 41-chromosome plant of Marquillo.
K.—Trivalents; metaphase of first division; 41-chromosome plant of Marquillo.
L.—Trivalents; metaphase of first division; 41-chromosome plant of Marquillo.



Stages: The meiotic divisions, microscrosporocytes; photomicrographs (× 1,500)

A.—Polyvalence, probably hexavalents; metaphase of first division; 41-chromosome plant; Marquillo.
B.—Polyvalence off the equatorial plane, number of chromosomes involved is unknown; 41-chromosome plant; Marquillo.
C.—Trivalents showing end constriction; metaphase of first division; 42-chromosome plant; Marquillo.
D.—Fragment above nucleolus; diakinesis of first division; 41-chromosome plant; Marquillo.
E.—Fragment; metaphase of first division; 42-chromosome plant; Marquis.
F.—Fragmentation; anaphase of first division; 42-chromosome plant; Marquillo.
G.—Fragmentation, same cell as F at different level; anaphase of first division; 42-chromosome plant; Marquillo.
H.—Fragmentation, same plant as F and G; different cell; anaphase of first division; 42-chromosome plant; Marquillo.
I.—Fragment and single chromosome which has undergone only reductional division; telophase of first division; Marquillo.
J.—Fragment and single chromosome which has undergone reductional and equational division; same cell as I; invert for comparison; telophase of first division; Marquillo.
K.—Fragment in both diads, same plant as I and J, different cell; metaphase of second division; Marquillo.
L.—Fragment, upper left microspore; tetrad; 42-chromosome plant; Marquis.

amounts of karyotin (pl. 1, A) in 12 of the 431 cells examined and of smaller amounts (pl. 3, A) of the same material in 28. This abnormality occurred in 2.8 and 6.5 per cent, respectively, of the microsporocytes.

In order to determine whether this phenomenon was due to the abnormal chromosome number of plant 407–15–17 the same studies were made with plant 407–12–24 and a plant of Marquis. In plant 407–12–24, 0.5 per cent of the 432 microsporocytes examined exhibited large extrusions and 1.9 per cent smaller extrusions. The plant of Marquis revealed 2 out of 540 microsporocytes showing extrusion of smaller karyotin clumps and one of the larger size. Extrusion of karyotin has been reported in the sporocytes of Crocus, Iris, Crepis, Oenothera, and other plants, according to Sharp (*16*).

NONORIENTATION OF BIVALENTS

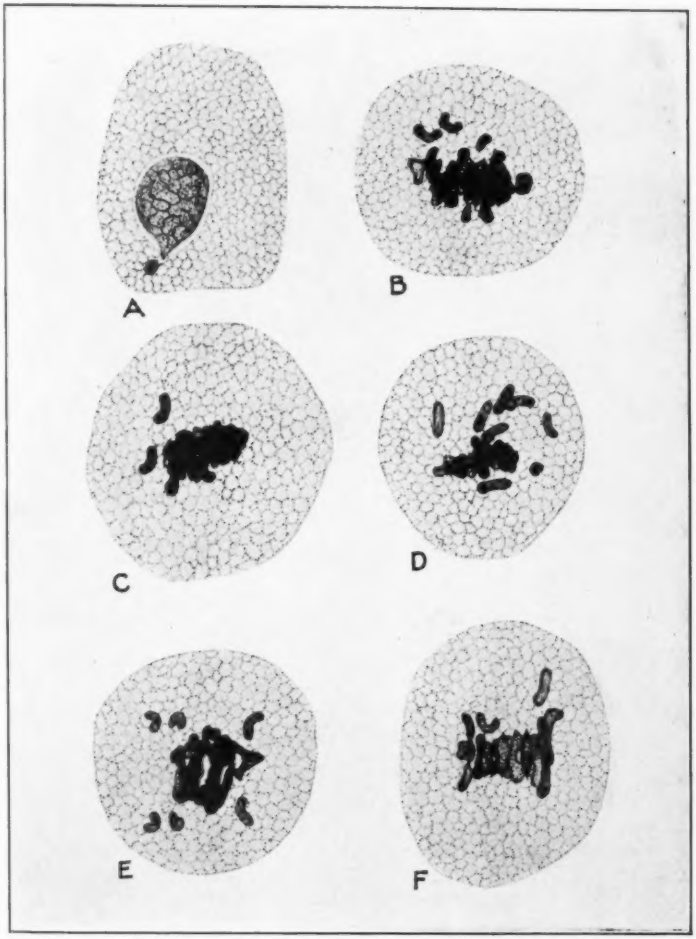
Between the diakinesis (pl. 2, D, and 5, A) and metaphase (pl. 1, B) stages there exists a transition period in which the nuclear cavity and nucleolus disappear and the chromosomes undergo transformation, losing the ragged and loose appearance shown in Plate 5, A, and becoming more definite in outline and compact, as shown in Plate 5, B. Examination of pollen mother cells showing this transition period does not indicate that the movement of the bivalents to the equatorial plane is a strictly methodical procedure. Some of the chromosomes may be at the equatorial plane while others are clearly defined in the surrounding cytoplasm. It seems, then, that at least in some cells all chromosomes do not undergo this transformation with the same degree of rapidity, nor do all seem to arrive on the equatorial plane at the same time. If such a supposition is true, it is to be expected that in some cases bivalent chromosomes should be found off the equatorial plane when all the other chromosomes are lined up and ready to undergo disjunction.

C and D of Plate 1 are photomicrographs of microspores of Marquillo culture 407–12–24 and Marquis culture 225–3 which exhibit this condition. As many as three bivalents in one cell have been found off the equatorial plane. This phenomenon has been termed nonorientation of bivalents. In these studies on nonorientation, only those cells showing bivalents not on the equatorial plane when the remaining chromosomes were definitely oriented and about to undergo disjunction have been considered as showing this phenomenon. Plate 1, B, is a side view of a Marquillo pollen mother cell showing all chromosomes lined up regularly on the equatorial plane. The frequency of the occurrence of nonorientation is of interest. An average of 10.8 ± 0.68 per cent of the 2,830 Marquillo microsporocytes studied showed nonorientation of bivalents and an average of 6.9 ± 0.49 per cent of the 3,327 Marquis microsporocytes revealed this aberration. The lowest percentage found in any plant of Marquillo was 3.8 and the highest was 28.6 per cent. The range in Marquis was from 1.8 to 21.4 per cent.

The question as to the subsequent behavior of nonoriented bivalents is important in connection with the point whether they result in the formation of micronuclei in the microspores. It seems possible that such bivalents might reach the equatorial plane late and undergo disjunction. If such were the behavior of these chromosomes, it would be expected that they could be found undergoing disjunction

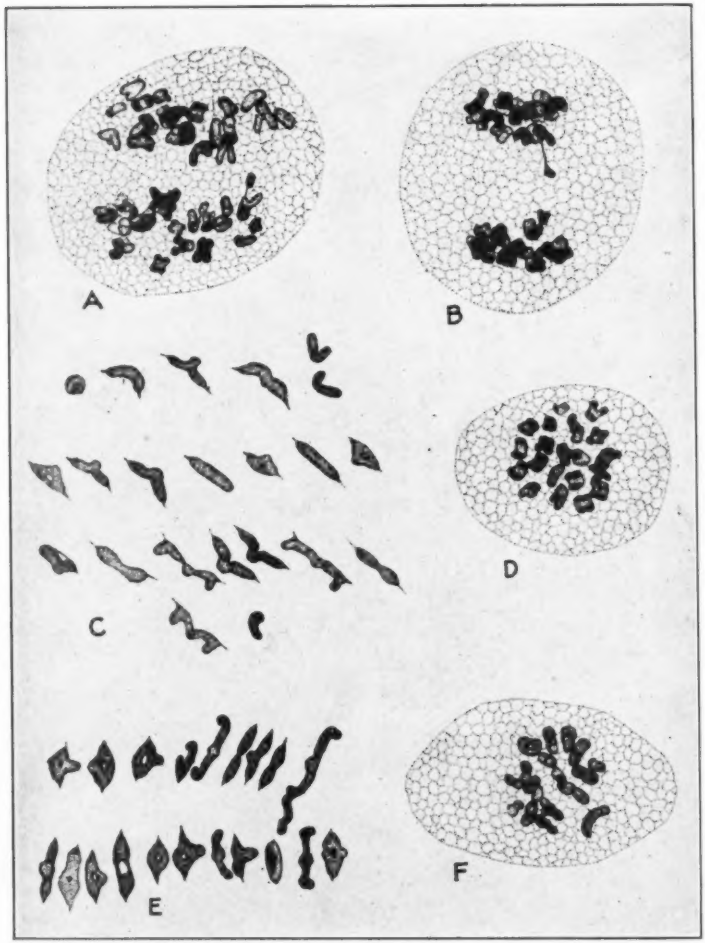
during the anaphase of the first division in some of the microsporocytes. No well-differentiated cases of late disjunction of a bivalent were found. However, there were only a few microsporocytes in the proper stage for detecting this phenomenon, and as nonorientation occurred in only 10.8 per cent of the Marquillo microspores and in only 6.9 per cent of those of Marquis, bivalents undergoing late disjunction on the equatorial plane would not necessarily be found even though they were occurring. That a differentation in the time of disjunction does occur is shown by Plate 3, E. At the right of the achromatic figure of this pollen mother cell are shown two bivalents. The chromosomes of one pair have pulled apart and are well started toward their respective poles, while the members of the other pair are still closely conjugated. It does not seem that the condition noted in Plate 3, E, would give rise to lagging chromosomes and hence micronuclei.

There also remains the possibility that these bivalents undergo disjunction even though they are not on the equatorial plane. B and C of Plate 3, are camera-lucida drawings offering evidence in support of this supposition. The two chromosomes in the upper left hand corner of Plate 3, B, are believed to have been loosely paired homologous chromosomes which have separated early. This conclusion is based on the fact that the two chromosomes closely resemble each other both in size and form and are in the same plane as regards their orientation on the achromatic figure. That this assumption is probably correct is further confirmed by the fact that the phenomena described are characteristics of early divided pairs whose members are on opposite sides of the main group of chromosomes as is shown in the same cell (pl. 3, B) and in a different microspore of the same plant (pl. 3, E). In contrast to these chromosomes are the nonconjugated chromosomes of Plate 3, D, which show no relationship to each other as regards their orientation in the achromatic figure. Plate 3, C, is also a camera-lucida drawing showing two chromosomes which it is believed are homologous mates that have disjoined off the equatorial plane. Since the disjoining of these two chromosomes, the lower has moved toward the lower pole and attained a position on the edge of the main group. In this particular cell it seems logical to suppose that the two chromosomes in question would reach their respective poles and be included in the reconstructed nuclei as they would have done had they lined up with the other bivalents. Other cells show this same phenomenon with the chromosomes in various positions. If this assumption that the members of bivalents can separate off the equatorial plane is correct, pairs should be found starting to show disjuntion. Plate 1, F, which is a photomicrograph of a microsporocyte of Marquis, shows two chromosomes paired end to end which are apparently undergoing disjuntion, being connected by only a small thread of chromatin. Two widely separated chromosomes in the same plane and on the same side of the equatorial plane are shown in Plate 1, E. This figure is also a photomicrograph of a microsporocyte of Marquis. The nonoriented bivalent of Plate 1, C, offers further evidence. This chromosome has undergone complete transformation in form, as can be seen from an examination of the photomicrograph. That its members are undergoing disjunction at the same time as the bivalents on the equatorial plane also seems probable as the free ends of the members are oriented toward opposite poles. The evidence supports the



Stages in the meiotic divisions, microsporocytes; camera-lucida drawings (× 2,000)

A.—Extrusion of karyotin; early leptotine stage; 41-chromosome plant; Marquillo.
B.—Predisjunction and nonorientation; metaphase of first division; 42-chromosome plant; Marquillo.
C.—Predisjunction and nonorientation; metaphase of first division; 42-chromosome plant; Marquillo.
D.—Nonconjunction, eight univalents; metaphase of first division; 42-chromosome plant; Marquillo.
E.—Predisjunction, three bivalents; metaphase of first division; 42-chromosome plant; Marquillo.
F.—Trivalents, two univalents; metaphase of first division; 41-chromosome plant; Marquillo.



Stages in the meiotic divisions, microsporocytes; camera-lucida drawings (× 2,000)

A.—Fragmentation; 42 chromosomes; same cell as Plate 2, F and G; anaphase of first division; Marquillo.
B.—Fragmentation; 42 chromosomes; same cell as Plate 2, H; anaphase of first division; Marquillo.
C.—20 bivalents, 1 single, drawn from side view; metaphase of first division; Marquillo.
D.—21 chromosomes, end view; anaphase of first division; Marquillo.
E.—19 bivalents, 1 trivalent, drawn from side view; metaphase of first division; Marquillo.
F.—20 bivalents, 1 univalent showing end constriction, end view; metaphase of first division; Marquillo.

supposition that the chromosome pairs at a certain period in transformation and development disjoin regardless of whether they are on the equatorial plane. It seems that the time at which this stage of development is reached may be correlated with the strength of association between the conjugants as exhibited by the type of pairing. For a discussion of the types of conjugation, which is not within the scope of this paper, see Aase (*1*).

Whether the nonoriented bivalents divide later than the oriented bivalents is of equal importance. It is doubtful whether the nonoriented bivalent shown in Plate 1, D, would disjoin as soon as the chromosome pairs on the equatorial plane, as it does not appear to have completely undergone the transformation that occurs between the diakinesis and metaphase stages of the first division. Although somewhat more regular in outline, it still is similar in form to some of the chromosomes in Plates 2, D, and 5, A. Then, this chromosome pair has the appearance of being a lagger in both transformation and orientation. If such is the true interpretation, this bivalent would not be expected to divide as soon as the chromosomes on the equatorial plane. Plate 1, G, may furnish some information on this problem. This photomicrograph of the telophase of the first division shows one univalent which has not undergone the second division and two separated chromosomes resulting from a univalent which has divided equationally. As shown here, chromosomes resulting from an equational division can be distinguished easily from these originating from a disjunctional division. Also, as is shown by the upper chromosome of Plate 1, G, the nonequationally divided chromosomes of this stage clearly show the split for the second division. It will be noted also that all three chromosomes are in the same plane, which strongly suggests that they represent separated homologous chromosomes which have first undergone a reductional division and then the lower resultant chromosome has undergone an equational division. The disjunction of the bivalent must have occured later than that of those giving rise to the nuclei at the poles, otherwise the nondivided chromosome would be expected to have been included with the main group at the pole. Also, it seems logical to assume that the bivalent giving rise to the lagging chromosome was not oriented on the equatorial plane at the time disjunction occurred, because if it had been both resultant daughter chromosomes would probably have exhibited the same behavior. On the other hand, if disjunction had taken place nearer the pole to which the undivided single is in proximity, the other member as it traveled toward the opposite pole may have been expected to undergo the equational division that has actually occurred. That this is an equational division and not a reductional division may be readily determined by a comparison between it and the nonoriented pair in Plate 1, C. That the upper chromosome of the three in Plate 1, G, has not undergone the equational division may be determined by comparing it with the chromosomes at the poles in Plates 2, F, and 4, A, and with the equationally divided lagging chromosomes in Plate 5, C. Comparatively speaking, the condition described can be found frequently in both Marquis and Marquillo. Plate 2, I–J, are photomicrographs of the same microsporocyte of Marquillo taken at different levels showing about the same condition as Plate 1, G. To have the orientation of the two photomicrographs comparable either one or the other should be inverted. A chromosome which has not

divided equationally is shown near the upper pole in Plate 2, I, whereas Plate 2, J, shows two chromosomes which have divided equationally. It seems highly probable that by dividing late nonoriented bivalents may give rise to the condition noted in Plate 1, G, and finally to micronuclei.

NONCONJUNCTION AND POLYVALENCE

From the foregoing results it seems that nonorientation of bivalents can account for some of the micronuclei occurring in the young microspores of Marquillo and Marquis. Other irregularities occur in the microsporogenesis of these two varieties and may be expected to have some influence on the prevalence of micronuclei. Univalents were found to be present, during metaphase of the first division, in some of the pollen mother cells of 42-chromosome plants of both Marquis and Marquillo. Evidently, for some reason or other, chromosomes which are usually conjugated at this stage of meiosis were univalent. This condition is termed "nonconjunction," and is illustrated in Plate 3, D, which is a camera-lucida drawing of a microsporocyte of Marquillo, culture 407–17–13. Frequently, cells having a single chromosome also showed three chromosomes united rather than the normal number of two. (Pl. 1, K–L; pl. 3, F.) In still other cells higher numbers were involved in a single union. This phenomenon was termed polyvalence. Nonconjugation and polyvalence are discussed together because of the relationship that may exist between the two phenomena.

It is not difficult to distinguish between nonconjunction (pl. 3, D) and predisjunction (pl. 3, E) when the predisjoined members are on opposite sides of the equator. However, when they are both on the same side of the main group of dividing chromosomes they can be distinguished only by their orientation with reference to the achromatic figure, as is shown by a comparison of Plate 3, C and D, the former being considered a case of predisjunction. With the possible exception of plant 225–5 of Marquis, the phenomenon of the predisjoining members being on the same side of the equatorial plane was infrequent as compared with the occurrence of nonconjunction.

TABLE 2.—*Frequency, in percentage, of the occurrence of nonconjunction and polyvalence, and the number of univalents involved in microsporocytes of Marquillo and Marquis*

Variety	Microspores showing—		Cells showing the indicated number of singles among those exhibiting nonconjunction						
	Nonconjunction	Polyvalence	1	2	3	4	5	6	8
Marquillo	6.1±0.44	1.4±0.27	50.0	36.3	6.3	2.5	3.1	1.3	0.6
Marquis	7.7± .93	.4± .10	78.3	18.8	1.8	1.1			

Table 2 gives the average percentage frequency of the occurrence of nonconjugation with the number of chromosomes involved and the average percentage frequency of polyvalence in the different cultures of Marquillo and Marquis.

An average of 6.1 ± 0.44 of the microsporocytes of Marquillo showed nonconjugation and 7.7 ± 0.93 per cent of those of Marquis. The difference between the two varieties is not statistically significant.

The range of nonconjunction in Marquillo was from 0 to 13.0 per cent and in Marquis from 2.3 to 40.2 per cent. The number of univalent chromosomes per cell in those showing nonconjunction ranged from 1 to 8 in Marquillo and from 1 to 4 in Marquis. The failure of homologous chromosomes to conjugate, for some unknown reason, would account for those cases where the number of univalents was even. If such were the correct explanation, 40.7 per cent of those cells of Marquillo showing nonconjunction would be accounted for and 19.9 per cent of those of Marquis. The possibility that these univalents may be due to very weak synapsis followed by disjunction of the homologous chromosomes before or during early metaphase must not be overlooked. This will be discussed further in connection with plant culture 225–5 of Marquis which showed 40.2 per cent of nonconjunction.

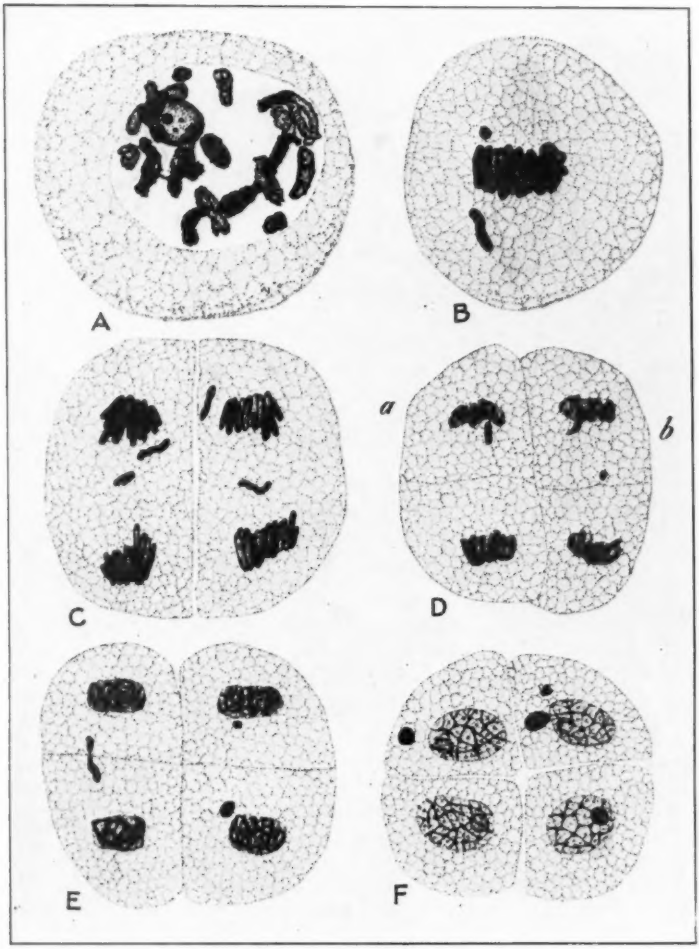
Even though 40.7 per cent of the cases of nonconjunction in Marquillo and 19.9 per cent of those of Marquis can be accounted for by the above explanation, there still remains 59.3 per cent and 80.1 per cent of the cases to be explained. It seems that the phenomenon of polyvalence may be responsible for a part of the nonconjunction found. Plate 1, K–L, shows photomicrographs of trivalents, and it is believed that six chromosomes are involved in the conjugation shown in Plate 2, A. It was impossible to determine exactly how many chromosomes were united in the polyvalence shown in Plate 2, B. It seems that three chromosomes are joined end to end in the microsporocyte illustrated in Plate 2, C. The joined chromosomes extended over and beyond the equatorial plane and curved back again, the lower part not being in focus. Two other cases of trivalents of the type illustrated in Plate 1, K, were clearly distinguishable in this cell and there were three univalents off the equatorial plane. These cases of trivalents occurring in one cell and accompanied by univalents were found in a 42-chromosome plant. All the other cases illustrated were found in 41-chromosome plants. The microsporocyte illustrated by a photomicrograph in Plate 1, K, is shown as a camera-lucida drawing in Plate 3, F. There are two univalent chromosomes, as would be expected because of the trivalent association and because of the fact that this is a 41-chromosome plant. It should be added here that in the majority of the cells showing trivalence it was not possible to locate an accompanying single. However, this does not preclude the possibility of the single being present, as it might have been obscured in the main group of chromosomes, or it might have formed another polyvalent association. The frequency of the occurrence of trivalents and tetravalents for Marquillo and Marquis is given in Table 2. Only two cases of other polyvalent associations were found. In Marquillo, on an average, 1.4 per cent of the cells examined showed polyvalence, and 84.4 per cent of the number showing this phenomenon exhibited trivalents and the remaining 15.6 per cent tetravalents. In Marquis only 0.4 per cent of the pollen mother cells examined during metaphase revealed unions of more than two chromosomes. It does not seem that the amount of polyvalence exhibited by either Marquillo or Marquis is sufficient to account for 59.3 and 80.1 per cent, respectively, of cases of nonconjunction, which is the amount not accounted for by failure of homologous chromosomes to pair. Then it seems that either some of the univalents, as such, are hidden by the bivalents or else the

polyvalent nature of some of the conjugants is obscured. The small correlation coefficient between polyvalence and nonconjunction of +0.28 substantiates the conclusion that the amount of polyvalence discernible does not account for a large percentage of the cases of nonconjunction. According to Fisher's (*2*) table for small numbers, this correlation is not statistically significant. However, it should be noted that a high correlation could not be expected because it is not likely that all the cases of trivalents were discernible and also because a relatively high proportion of the nonconjunction noted probably was due to failure of homologous chromosomes to undergo synapsis.

Plant culture 225–5 of Marquis is of special interest because it exhibited nonconjunction in 40.2 per cent of the microsporocytes examined. E and F of Plate 1 are photomicrographs of pollen mother cells of culture 225–5. Plate 1, F, shows very weak conjugation between the bivalents not on the equatorial plane. Plate 1, E, shows a still greater separation between two single chromosomes that may have been paired. Of the 179 pollen mother cells examined in this plant 35 revealed one single chromosome, 31 two singles, 3 three singles, and 3 four singles. It seems as though in this particular plant two homologous chromosomes must have lost their affinity for each other, or perhaps that statement should be modified by saying the greater part of their affinity, as weak conjugation was noted in a number of cases. The occurrence of a high percentage of the microsporocytes of this plant showing nonconjunction might be expected to be reflected in a high percentage of the microspores showing micronuclei. Out of 552 immature pollen grains of other spikelets of the same plant examined, only 1.1 per cent showed micronuclei as compared to an average of 0.8 per cent for the 27 Marquis plants studied. Three hundred well-stained immature pollen grains from the same spikelet were studied and micronuclei were found in only 1.2 per cent of them. These results indicate that either the high percentage of nonconjunction noted is not common to all the microsporocytes of plant 225–5 or else the univalent chromosomes are reaching the poles during meiosis and being included in the macronuclei of the microspores. The former hypothesis seems the most plausible, as is shown in a report on culture 223, in a later section of this paper.

PREDISJUNCTION

Only those cases in which both chromosomes were on opposite sides of the equatorial plane (pl. 3, B and E) were included in the determinations of the percentage of predisjunction. The microsporocytes of Marquillo showed 6.3 ± 0.69 per cent of this phenomenon and those of Marquis 2.8 ± 0.31 per cent. The difference between the two varieties is statistically significant, as can be seen from an examination of the probable errors. The range in Marquillo was from 0 to 18.5 per cent and in Marquis from 0 to 7.9 per cent. The number of bivalents involved in predisjunction ranged from 1 to 3 in both Marquillo and Marquis. It is difficult to determine the significance of predisjunction, but it seems doubtful whether this condition gives rise to micronuclei, as the chromosomes arriving at the poles early probably would be joined later by the main group.



Stages of meiotic division, microsporocytes; camera-lucida drawings (× 2,000)

A.—Fragment, above nucleolus, same cell as Plate 2, D; diakinesis of first division; 41-chromosome plant; Marquillo.

B.—Univalent and fragment, same plant as A; metaphase of first division; 41-chromosome plant; Marquillo.

C.—Lagging univalents, same plant as A and B; late anaphase of second division; 41-chromosome plant; Marquillo.

D.—Lagging chromosome (*a*) and fragment (*b*); same plant as A, B, and C; telophase of second division; 41-chromosome plant; Marquillo.

E.—Fragmentation, fragment, and micronucleus; same plant as A, B, C, and D; telophase of second division; 41-chromosome plant; Marquillo.

F.—Fragment and two micronuclei; same plant as A, B, C, D, and E; tetrad; 41-chromosome plant; Marquillo.

FRAGMENTATION

Irregularities other than those involving entire chromosomes were noted during microsporogenesis of Marquillo and Marquis. Plant 407–15–17, a 41-chromosome plant, showed during metaphase, in addition to the univalent chromosome, a small lagging fragment. It was possible to find the fragment of a chromosome not lined up on the equatorial plane in 70.2 per cent of the microsporocytes in the metaphase stage of the first division. (Pl. 5, B.) The fragment was not distinguishable in the remaining 29.8 per cent of the cells. It may have become obscured by having formed unions with other chromosomes or by having lined up on the equatorial plane. Plate 4, F, is an end view of the metaphase showing 20 pairs and 1 single, and offers some evidence for the hypothesis that the fragment may form unions with other chromosomes. The single chromosome appears constricted at one end, and as the fragment was not visible in this microsporocyte it seems plausible that the constriction might mark the point at which the fragment had become attached. A clearer case of an end constriction is shown in Plate 2, C, which is a photomicrograph of another plant of Marquillo.

Good preparations of microsporocytes of plant 407–15–17 in other stages of development were available also. The fragment was discernible in 83.9 per cent of the 93 cells examined in the diakinesis stage. It was usually located near the periphery of the nuclear cavity, as is shown in the upper portion of Plates 2, D, and 5, A, which are a photomicrograph and a camera-lucida drawing of the same cell. The presence of the fragment in the diakinesis and metaphase stages raises the question as to whether or not it can be found in the young microspores. Thirty-one and three-tenths per cent of the 198 microspores examined in the tetrad stage showed a small clump of chromatin. (Pl. 5, D–F.) In some instances (pl. 5, F) the small clump was accompanied by a larger micronucleus and in others by the macronucleus only. The distinction between the micronuclei and the small clumps of chromatin was made on the basis of size. Evidence that the two could be differentiated is obtained by comparing the percentage of microspores of other 41-chromosome plants showing micronuclei with that of plant 407–15–17. The percentage of microspores showing micronuclei in plant 407–15–17 was 30.3, and the average for six 41-chromosome plants was 23.4. The percentage for plant 407–15–17 is already somewhat higher than expected on the basis of other 41-chromosome plants and would be still higher if the percentage of microspores showing small clumps of chromatin in the cytoplasm were added to it. This, then, is further proof that the fragmented piece of chromosome seen in the diakinesis and metaphase stages is responsible for the small clumps of chromatin found in the microspore.

Second divisions were not available in plant 407–15–17, but plant 407–7–5 showed fragments in the second division. (Pl. 2, K.) Seventy-five microsporocytes in the metaphase of the second division were studied: 60 showed 1 fragment in each member of the diad, 6 showed 2 fragments in one member and none in the other, and 1 showed 2 fragments in each member. In the one case having 2 fragments in each member of the diad, the appearance in both is as if 1 may have divided to form 2. The position of the fragments

varied; being sometimes close to the equatorial plane and at other times some distance from it. A few telophases of the first division were available. Plate 2, I–J, shows photomicrographs of the same cell taken at different levels, and in order for the poles to correspond either the one or the other should be inverted. Both of the fragments were present and were in proximity to each other, appearing as though the two fragments had arisen from a division of one. Metaphases of the first division and microspores were not available.

It would be interesting to know the period or periods in the life cycle of the plant at which these fragments arise. It is not possible to say definitely at what stage fragmentation took place in the Marquillo cultures 407–15–17 and 407–7–5, but that fragmentation can take place during the anaphase of the first division is shown by Plate 2, F–H. The photomicrographs in Plate 2, F and G, are of the same microsporocyte taken at different levels. At the upper right side of the former is a chromosome, one of the chromatids of which reveals the beginning of a break. A chromosome in the lower right end of Plate 2, G, shows the same phenomenon in a more advanced condition, and Plate 2, H, represents a still more advanced stage with the fragmented piece at the equatorial plane. The chromatid involved seems to be breaking at approximately the center, as is shown by Plate 2, F. Camera-lucida drawings of the two microspores involved (pl. 4, A and B) show the chromosome number of this plant (407–7–13) to be 42. These two cells were the only two microsporocytes of this plant exhibiting fragmentation. Also, 125 pollen mother cells in the metaphase stage of the first division were examined without evidence of fragmentation being found, proving that it occurred as noted for the first time during anaphase of the first divisions.

That fragmentation also occurs in Marquis is shown by Plate 2, E and L; the former being the metaphase of the first division and the latter the tetrad stage, showing a fragment in the cytoplasm of the upper left-hand microspore.

No definite attempt was made to determine the frequency of fragments in Marquillo and Marquis, but any fragmentation noted while the microsporocytes of these varieties were being studied for other abnormalities was recorded. Twelve of the 32 plants of Marquillo examined and 8 of the 27 plants of Marquis were recorded as showing some degree of fragmentation. From these figures it would seem that fragmentation is fairly common in both Marquillo and Marquis, but such a conclusion would be erroneous, as only one instance of fragmentation in any of the stages of a particular plant studied would have been recorded as the occurrence of fragmentation. That fragments could have been found in a relatively large proportion of the plants and still be of infrequent occurrence is emphazised by the fact that the total number of cells studied, including both microsporocytes and microspores, would average somewhat over 600 per plant.

Fragmentation has been shown to take place during the anaphase of the first division. This could not directly explain the occurrence of fragments in the diakinesis and metaphase stages of the first division of plant 407–15–17, nor could it be expected to explain the large proportion of the microsporocytes of plant 407–7–5, which showed this phenomenon during the metaphase of the second division. The fact that the diakinesis and metaphase stages show fragmenta-

tion, together with its high frequency in the second division, indicates that in these plants the abnormality must have arisen in the somatic tissue or in the sexual cells of the preceding generation . If the latter supposition is correct, the behavior of the fragment may be likened to that of a univalent chromosome.

PROGENY OF SELECTED PLANTS

Progeny of three of the plants of Marquillo studied cytologically were grown in 1930 for the purpose of conducting further investigations. Culture 407–17–13 was grown because of the comparatively high percentage of the different aberrations found to occur during microsporogenesis. Three and five-tenths per cent of the microspores possessed micronuclei, 14.4 per cent of the microspores exhibited nonorientation, 18.8 per cent nonconjunction, and 16.0 per cent predisjunction. These are considerably above the averages for Marquillo, which were 2.8, 10.8, 16.1, and 6.3, respectively. In addition, the number of univalent chromosomes in the pollen mother cells showing nonconjunction varied from 1 to 8, the greatest range in any plant studied. The percentage of microsporocytes revealing polyvalence, was only 1 per cent above the average for Marquillo. Plate 3, B–E, shows camera-lucida drawings of aberrations found in the microspores of this plant. The progeny of culture 407–12–24 were included for further studies because, in general, its microsporocytes exhibited fewer aberrations than the average for all the plants of Marquillo. The regularity of the alinement of its chromosomes on the equatorial plane during metaphase of the first division is shown in Plate 1, B. Of the microspores examined, 2.6 per cent revealed micronuclei, and of the microsporocytes examined, 6.0 per cent showed nonorientation, 6.7 per cent nonconjunction, 4.4 per cent predisjunction, and 0 per cent polyvalence. Culture 407–12–3 of Marquillo was included because of its being a 41-chromosome plant.

The number of chromosomes in the 10 progeny of culture 407–17–13 was determined on the basis of the percentage of microspores showing micronuclei, with the exception of plants 223–10 and 223–4 the number of whose chromosomes was determined by counts made in the anaphase of the first division. That this is an accurate method of determining whether univalent chromosomes are present is shown by an examination of Table 3. As this table shows, the percentage of microspores of 41-chromosome plants exhibiting micronuclei varied only from 22.0 to 25.3 per cent. As all the plants of Marquillo studied had either the normal number of chromosomes, 21 pairs, or 20 pairs and 1 single, or 19 pairs and 2 singles, it seems safe to conclude that all the progeny of culture 407–17–13, with the exception of 223–10, had the normal number.

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TABLE 3.—*Chromosome number and behavior during microsporogenesis of the progeny of a 41-chromosome plant of Marquillo, 407–12–3*

Culture No.	Number of chromosomes	Microsporocytes showing indicated number of singles during metaphase						Total microsporocytes	Microsporocytes showing univalents		Trivalent microsporocytes[b]	Tetravalent microsporocytes[b]	Microsporocytes		Total microspores	Microspores showing micronuclei	
		0	1	2	3	4	5		Number	Per cent			Total	Per cent		Number	Per cent
222–1[a]	40	.....	5	102	5	8	.....	120	120	100.0	4	0	4	3.3	462	196	42.4
222–3	41	5	71	1	.....	.....	.....	77	72	93.5	3	1	4	5.2	537	131	24.4
222–5	41	11	218	8	24	.....	1	262	251	95.8	5	0	5	1.9	644	163	25.3
222–6	41	20	186	1	5	.....	.....	212	192	90.6	2	1	3	1.4	564	124	22.0
222–7	41	16	48	.....	.....	.....	.....	64	48	75.0	2	0	2	3.1	518	114	22.0
222–8	41	32	234	2	7	.....	.....	275	243	88.4	0	1	1	.4	460	105	22.8
222–9	41	4	148	2	3	.....	.....	157	153	97.5	6	2	8	5.1	475	113	23.8
Total	.....	88	905	14	39	.....	1	1,047	959	540.8	18	5	23	17.1	3,198	750	140.3
Percentage of total	.....	8.4	86.4	1.3	3.7	.....	.1	100.0	.....	.....	78.3	21.7	100.0	.....	.....	.....	.....
Average	.....	14.7	150.8	2.8	9.8	.....	.....	174.5	159.8	90.1±2.15	.....	.....	.....	2.9±.50	533.0	125	23.4±.24

[a] Omitted from calculations.
[b] Number of microsporocytes showing some trivalent and tetravalent associations, respectively.

Counts of plant 223–10, made from the anaphase of the first division, showed without exception that this plant had 41 chromosomes. This finding is further substantiated by an examination of microsporocytes in the metaphase of the first division and others in the tetrad stage. Of 119 microsporocytes in the metaphase of the first division 108 showed a univalent chromosome, 4 three univalents, and 7 no univalents. In 2 of the 7 microsporocytes not showing any single chromosomes two cases of trivalents such as noted in Plate 4, E, were clearly visible. These results show the chromosome behavior during the metaphase of the first meiotic division to be characteristic of the 41-chromosome plants already described and those to be discussed later. Twenty-one and seven-tenths per cent of the microspores in the tetrad stage exhibited micronuclei, which amount is very close to the 23.4 per cent exhibited by the 41-chromosome progeny of a 41-chromosome plant, culture 407–12–3. These results are important in showing that a 41-chromosome plant may arise from one having 42 chromosomes. From the foregoing discussion on chromosomal aberrations it is easy to visualize how such a 41-chromosome plant could originate. If a normal 21-chromosome male gamete fertilized a female gamete having only 20 chromosomes, owing to nonorientation or some of the other previously reported aberrations, a 41-chromosome zygote would result. As the spikes of this plant were not covered the previous year there still remains the possibility that the 41-chromosome plant might have resulted from pollination of a normal flower with stray pollen from another 41-chromosome plant. It can be shown from data given on 41-chromosome plants that the chances of the 41-chromosome plant being the result of chromosomal aberrations are fifty times greater than that of its being due to cross-pollination.

Another interesting abnormality was found in one of the progeny of culture 407–17–13. While making counts of the young microspores of plant 223–4 to determine the percentage that possessed micronuclei, it was noticed that the upper pollen sac of plant 223–4 was averaging strikingly higher in percentage of microspores showing one or more micronuclei than was the lower pollen sac of the same anther. It will be remembered that all spikelets were cut longitudinally and therefore would be expected to show a considerable number of microspores in each section. Counts were made in the four sections showing the two pollen sacs; the results obtained are recorded in Table 4.

TABLE 4.—*Variations between different pollen sacs of the same anther, in the percentage of microspores showing micronuclei, plant of Marquillo, culture No. 223-4*

Section No.[a]	Microspores in upper pollen sac			Microspores in lower pollen sac		
	Total	Showing micronuclei		Total	Showing micronuclei	
		Number	*Per cent*		*Number*	*Per cent*
1	56	11	19.6	42	0	0
2	68	22	32.4	54	1	1.9
3	98	17	17.3	65	3	4.6
4	75	15	20.0	41	0	0
Total	297	65		202	4	
Average			22.3±2.02			1.6±0.64

[a] On slide No. 51.

In the upper pollen sac 22.3 ± 2.02 per cent of the 297 microspores counted revealed micronuclei, whereas only 1.6 per cent of the microspores counted in the lower pollen sac exhibited this condition. The percentage of microspores showing micronuclei is very close to the average of 23.4 ± 0.24 shown by 41-chromosome plants. This strongly suggests that the microsporocytes giving rise to the microspores must have possessed one unpaired chromosome, probably 20 bivalents plus 1 univalent. Since the lower pollen sac did not show this high percentage of abnormalities, the mutation giving rise to the univalent chromosome must have occurred some time during the development of the young anther, after the tissue giving rise to the pollen sacs had become differentiated, perhaps in the first archesporial cell. There were only 16 microsporocytes in the metaphase available, and only 1 showed a single chromosome. There were a number of microsporocytes in the diad stage, but lagging chromosomes were not any more frequent that in 42-chromosome plants of Marquillo. These results indicate that the high percentage of abnormalities is limited to the upper pollen sac of this one anther.

The average percentage of abnormalities in the microsporocytes of the progeny of culture 407–17–13 was 4.7 ± 1.44. The range in variability was from 0.5 per cent in plant 223–12 to 21.7 per cent in plant 223–10 and the coefficient of variability was 143.0.

The progeny of culture 407–12–24 was grown because in general this plant exhibited a lower percentage of the different aberrations than the average of the Marquillo plants studied. As determined by the percentage of microspores showing micronuclei, all of the 16 progeny studied possessed the normal number of chromosomes. It will be remembered that culture 407–12–24 exhibited micronuclei in 2.6 per cent of the microspores. The average percentage of the microspores of the progeny exhibiting micronuclei was 2 ± 0.13, and the range of variability was from 1.1 per cent to 3.4 per cent. The coefficient of variability was 39.7. These data show that the average percentage of micronuclei was only 0.6 of one per cent below that of the parent grown the previous year. Also, it is of interest to compare the progeny of culture 407–17–13 and 407–12–24. The difference of 2.7 per cent between the percentage of microspores showing micronuclei is not significant because of the high probable error of culture 407–17–13. The range of variability in progeny of culture 407–17–13 is 18.9 per cent higher than that for the progeny of culture 407–12–24. These data are based upon an examination of 5,610 microspores of the 10 progeny of culture 407–17–13 and 9,298 of the 16 progeny of culture 407–12–24.

Of the seven progeny of culture 407–12–3 studied, it was possible to obtain counts from the anaphase of the first division in all plants except 222–7. In this plant counts were made only from the metaphase of the first division. A summary of the data obtained is given in Table 3.

All plants were found to have 41 chromosomes, except 222–1, which possessed only 40, consisting of 19 pairs and 2 singles. Of the 120 microsporocytes of plant 222–1 examined, 102 showed two singles not on the equatorial plane, 5 one single, 5 three singles, and 8 four singles. These figures show that 85 per cent of the microsporocytes had 2 univalent chromosomes, which compares very favorably with the data obtained for the six 41-chromosome plants. In these plants

86.4 per cent of the 1,047 microsporocytes examined showed 1 univalent chromosome. Also, 42.4 per cent of the microspores of plant 222–1 possessed micronuclei, as compared with 23.4 ± 0.24 per cent for the 41-chromosome plants. These results support the counts showing plant 222–1 to have 40 somatic chromosomes, composed of 19 bivalents and 2 univalents. Table 3 shows that the data taken individually on the six remaining plants do not vary greatly, which is strong evidence supporting the counts that show these plants to be composed of 20 bivalent chromosomes plus 1 univalent.

These results are not those to be expected on the basis of random distribution of the single chromosome during meiosis of the microsporocytes and the megasporocytes. If the single chromosomes went at random to the poles during the first division, 50 per cent of the gametes would be expected to contain 21 chromosomes and the remaining 50 per cent would be expected to have 20 chromosomes. Random mating and survival of all the progeny would give a ratio of one 42-chromosome plant; two 41-chromosome plants; one 40-chromosome plant. Instead of this ratio six 41-chromosome plants and one 40-chromosome plant were obtained. The constitution of the 40-chromosome plants, according to theory, should be 20 bivalents and no univalents. The 40-chromosome plant was composed of 19 bivalents plus 2 singles, which makes it appear as though it should be classified among those having 41 chromosomes. The loss of a chromosome through nonorientation or some of the other aberrations noted in 42-chromosome plants would account for this condition of 19 pairs and 2 singles.

The behavior of the single chromosome during meiosis is important as its determination would furnish information as to the manner in which the different aberrations noted during metaphase of the first division of 42-chromosome plants give rise to micronuclei in the microspores. An attempt was made to follow the single chromosome through the various stages of meiosis. It was possible to distinguish the single chromosome in 86.4 per cent of the metaphases of the first division. Counts were made of the number of chromosomes at the poles in 27 microsporocytes in the anaphase of the first division. In 17 of these the single chromosome had gone to the poles without dividing or lagging, as was evidenced by there being 20 chromosomes at one pole and 21 at the other. In the other 10 cells the univalent chromosome was found lagging and dividing. Therefore, in 63 per cent of the cells the single chromosome was not lost, whereas in 37 per cent it was lost. On this basis 18.5 per cent of the microspores would be expected to show a micronucleus and the expected ratio of 20:21 chromosome gametes would be 69:31. The studies of the second division were made on the microsporocytes having both members of the diad present, and the percentage of microsporocytes showing the lagging chromosome was determined on the basis of both showing it. In the metaphase of the second division only 1 out of the 18 microsporocytes examined exhibited lagging chromosomes in both metaphases and none exhibited a lagging chromosome in only one member of the diad. On this basis only 2.7 per cent of the microspores would be expected to possess micronuclei. The results obtained can be explained by assuming that the already equationally divided chromosomes are included on the equatorial plane with the others. This agrees with the conclusions of Watkins (*18*) that the

single chromosomes dividing in the first division are regained in the second. Of the 51 pollen mother cells studied in the anaphase or early telophase of the second division, 22 exhibited lagging chromosomes in both members. On this basis 21.6 per cent of the microspores in the tetrad stage would be expected to show a micronucleus and the expected ratio of 20:21 chromosome gametes would be 72:28. A study was made on 3,198 microspores, 750 or 23.4 per cent of which showed a micronucleus. On this basis the expected ratio of 20:21 chromosome gametes would be 74:26. These data are summarized in Table 5.

TABLE 5.—*Percentage of microsporocytes showing the univalent chromosome lagging or off the equatorial plane during microsporogenesis of 41-chromosome plants*

Stage of microsporogenesis	Microsporocytes			Expected percentage of microspores showing micronuclei	Expected ratio of 20:21 chromosome gametes
	Total	Showing univalents			
		Number	*Per cent*		
Metaphase of first division	1,047	905	86.4	21.8	72:28
Anaphase of first division	27	10	37.0	18.5	69:31
Metaphase of second division	18	1	5.6	2.7	53:47
Anaphase or early telophase of second division	51	22	43.1	21.6	72:28
Tetrad [a]	800	375	47.0	23.5	74:26
Back cross [b]				23.0	73:27

[a] Counts made from examination of young microspores and converted to microsporocyte basis.
[b] Ratio of 20:21 chromosome gametes in megaspores as determined by back crossing to a 42-chromosome plant was 73:27 (Nishiyama *14*).

Nishiyama (*14*) made a study of the ratio of 20:21 chromosome gametes by back crossing a 41-chromosome plant obtained from a cross of *Triticum polonicum*×*spelta* with *spelta*, and found that the ratio of 20:21 chromosome gametes was 73:27. He concluded from examination of Kihara's (*11*) and Watkins' (*17*) results that loss of chromosomes occurs with about the same frequency in microspore formation as in magaspore formation. If this assumption is correct, the results reported here are in very close agreement with those obtained by the back-cross method.

The data on the behavior of the univalent chromosome of 41-chromosome plants can be satisfactorily explained by the following hypothesis. The chances of the univalent chromosome undergoing the equational division in the first division are slightly less than those of its passing to the poles without dividing. In the latter case the univalent chromosome behaves normally and micronuclei are not formed in the microspores. In those cases in which the univalent chromosomes divide equationally in the first division the resulting daughter chromosomes are distributed to opposite diads and both are included in the achromatic figures but are not discernible. However, another division does not occur but instead these chromosomes lag and eventually round up to form the micronuclei found in 23.4 per cent of the microspores of 41-chromosome plants. It should be noted here that the percentage of microspores showing micronuclei due to the univalent chromosome, is probably in reality somewhat lower than that reported because of other chromosomal aberrations producing the same phenomenon.

Generally it has been assumed by the various investigators that the reason for not being able to discern the univalent chromosome or chromosomes in all microsporocytes showing the metaphase of the first division is because of the other chromosomes obscuring it. The close agreement between the expected percentage of microspores showing micronuclei based on a study of the metaphase of the first division (Table 5) and that based on the anaphase or early telophase of the second division with the percentage actually found by counts made of the tetrad stage indicates that the univalent chromosome may not have been present in those cells in which it was not discernible. The extrusions of karyotin, previously described, would furnish the mechanism whereby it could have been eliminated during the early stages of sporogenesis.

The agronomic characters of the progeny of these cultures are reported in Tables 6 and 7.

Table 6 shows that Marquis surpassed culture 407–12–24 in number of spikes, percentage of fruitfulness, and height in inches, but was inferior in weight of seeds, percentage emerged based on number of seeds planted, and percentage matured based on the number of seeds planted. Marquis and culture 407–12–24 did not differ significantly in respect to their coefficients of variability. However, culture 407–12–24 had a higher coefficient of variability than Marquis as regards number of spikes and percentage of fruitfulness, but the reverse was true for weight of seed in grams and height in inches. Culture 407–17–13 without exception averaged higher in all six characters studied and in general showed a lower coefficient of variability. On the other hand, culture 407–12–3 averaged lower in all characters than any other culture studied with one exception. Marquis was lower in percentage of plants to emerge based on number planted. It will be remembered that culture 407–12–3 was a 41-chromosome plant culture 407–17–13 showed a high percentage of abnormalities during microsporogenesis, culture 407–12–24 exhibited a low percentage of abnormalities during microsporogenesis, and Marquis was planted for purposes of comparison. The progeny of the 41-chromosome plant possessed a higher coefficient of variability for every character studied. (Table 7.)

TABLE 6.—*Average values of statistical measures of the characters of selected lines of Marquillo and Marquis from measurements made on individual plants*

Variety or culture No.	Individuals	Height	Spikes	Fruitfulness	Weight of seed	Emergence based on number of seeds planted	Maturation based on number of seeds planted
	Number	*Inches*	*Number*	*Per cent*	*Grams*	*Per cent*	*Per cent*
Marquis	24	37.1±0.39	17.1±1.01	87.4±1.64	4.8±0.43	86.7	83.3
407–12–24	23	29.2± .17	13.4± .87	83.5±1.93	5.7± .48	92.0	84.0
407–17–13	15	34.4± .28	20.1±1.02	88.3±2.24	9.4± .74	100.0	93.8
407–12–3	7	25.6± .82	10.7±1.33	62.5±5.25	1.9± .33	88.9	80.0

TABLE 7.—*Coefficients of variability of characters of the progeny of selected lines of Marquillo and Marquis from measurements made on individual plants*

Variety or culture No.	Individuals	Height	Spikes	Fruitfulness	Weight of seed
	Number	*Inches*	*Number*	*Per cent*	*Grams*
Marquis	24	7.7±0.75	42.7± 4.86	13.7±1.36	65.8± 8.75
407-12-24	23	4.1± .41	46.0± 5.46	16.4±1.67	59.6± 7.75
407-17-13	15	4.6± .57	29.1± 3.87	14.5±1.82	45.3± 6.62
407-12-3	7	12.5±2.29	48.9±10.72	33.0±6.56	68.9±17.34

CORRELATIONS BETWEEN CYTOLOGIC ABERRATIONS

It seemed important to determine the relationships of the different aberrations to each other and if possible the influence that they may exert upon the characters of the plant. Since it was impossible to distinguish qualitative differences, these characters have been omitted from the discussion. All measurements of quantitative characters were made on individual plants and expressed on the plant basis. It is important to keep in mind that the correlations between abnormalities and plant characters calculated for Marquillo, with the exception of one correlation on fruitfulness, are not comparable with those of Marquis, the reason for this being that the characters correlated with aberrations in Marquillo are of the progeny of the plants studied cytologically, whereas those of Marquis are the characters of the plants studied cytologically. This would make the two sets of data differ in two essential points. (1) The microsporocytes studied give rise to the plants with whose characters their abnormalities are correlated and (2) the measurements are based on the average of many plants in Marquillo. In the case of Marquis the abnormalities of the microsporocytes are correlated with the characters of the plants producing them and of necessity the measurements are based on only one plant. However, the correlations between the different abnormalities found in Marquillo and the same correlations for Marquis are comparable.

For determining the statistical significance of coefficients of correlation, Fisher's (*2*) table giving values of the correlation coefficient for different levels of significance was used. Coefficients of correlation giving a P value of 0.05 were considered as being statistically significant. If N is 20 a correlation of 0.42 would be necessary to give a P value as low as 0.05. Fisher's tables have been used because the coefficients of correlation reported in this work are based upon small numbers. It is usual to measure the significance of the correlation r by employing the probable error obtained by the formula $Er = 0.6745 \frac{1-r^2}{\sqrt{n}}$. With small samples the value of r is often very different from the true value and consequently $1-r^2$ is in error, and also the distribution of r for high correlations in small samples is far from normal, so that tests of significance based on the Er determined from this formula may be misleading. For practical purposes by the use of Fisher's table these two errors are avoided.

Table 8 gives the simple and partial correlations obtained between percentage frequency of different aberrations occurring during microsporogenesis of Marquillo and Marquis. These correlations were calculated in an endeavor to determine the part that each of the

aberrations, nonorientation of bivalents, nonconjunction, and predisjunction played in causing the occurrence of the micronuclei found in microspores in the tetrad stage.

TABLE 8.—*Coefficients of correlation between percentage frequencies of different aberrations found occurring during microsporogenesis of Marquillo and Marquis* [a]

Marquillo			Marquis		
Simple	Partial	N	Simple	Partial	N
$r12+0.70$	$r12.34+0.72$	30	$r12+0.70$	$r12.34+0.70$	27
$r13+ .32$	$r13.24+ .17$	25	$r13+ .17$	$r13.42+ .15$	26
$r14+ .21$	$r14.32+ .20$	27	$r14- .14$	$r14.32+ .10$	25
$r23+ .12$	$r23.14+ .01$	25	$r23+ .12$	$r23.14- .24$	26
$r24- .05$	$r24.13- .24$	27	$r24- .25$	$r24.13- .23$	25
$r34+ .58$	$r34.12+ .54$	24	$r34- .31$	$r34.12- .30$	25
$R_{1.234}$ .75			.71		

[a] Key to numbers: 1 = micronuclei; 2 = nonorientation; 3 = nonconjunction; 4 = predisjunction. Levels of significance: According to Fisher's (t) tables for the values of the correlation coefficient for different levels of significance an N of 20 and r of 0.42 gives a P value of 0.05.

The only significant simple and partial correlations obtained in both Marquis and Marquillo were those between nonorientation of bivalents and micronuclei, the simple correlation being +0.70 in both Marquillo and Marquis, and the partial correlations being +0.72 and +0.70, respectively. Both the simple and partial correlations between nonconjunction and micronuclei in Marquillo and Marquis were positive but not statistically significant, being +0.32 and +0.17, respectively, for Marquillo and +0.17 and +0.15 for Marquis. The simple correlations between nonorientation and nonconjunction in both Marquillo and Marquis were +0.12 and the partial correlations were +0.01 and −0.24, respectively. None of these values are statistically significant.

These results indicate that nonorientation and nonconjunction are independent of each other, and such a conclusion is what would be expected from the cytologic studies. Such is not true, however, of the association between nonconjunction and micronuclei, as there seems to be no cytological basis for assuming that at least a part of the univalents found during metaphase of the first division should not give rise to micronuclei in the tetrads. Especially is this true since the univalent chromosome of 41-chromosome plants has been found to do so. Then the question arises whether the correlations found measure the true relationship existing between nonconjunction and micronuclei even though they are not statistically significant. The fact that all the correlations between these two aberrations in both Marquillo and Marquis are positive indicates that they are probably more important than the level of significance set up indicates. Therefore, considering both the cytologic studies and the coefficients of correlation it seems that the phenomenon of nonconjunction gives rise to micronuclei.

In the studies with Marquillo a simple correlation of +0.21 and a partial correlation of +0.20 were obtained between micronuclei and predisjunction. The corresponding correlations in Marquis were −0.14 and +0.10. The simple and partial correlations between nonconjunction and predisjunction were +0.58 and +0.54, respectively, in Marquillo and −0.31 and −0.30 in Marquis. The correla-

tions between nonconjunction and predisjunction are statistically significant in Marquillo but are not in Marquis. It seems doubtful whether predisjunction is instrumental in the production of micronuclei in the tetrads. This is in agreement with the cytologic evidence.

From a consideration of all available data it seems logical to conclude that nonorientation is most prominent in the production of micronuclei, but that nonconjunction also plays some part.

CORRELATIONS BETWEEN PERCENTAGE FREQUENCY OF DIFFERENT ABERRATIONS AND STATISTICAL MEASURES OF PLANT CHARACTERS

The coefficients of correlation were used to determine the relationship existing between the abnormalities occurring during microsporogenesis and plant characters. The data may be separated into two distinct groups. In the first group are included the coefficients of correlation between cytologic aberrations and the statistical measure of characters of the plants which produced them, and in the second group are given the coefficients of correlation between cytologic abnormalities and the statistical measures of the characters of the progeny of the plants in which these aberrations were studied. Only quantitative characters were used in the studies as no differential qualitative characters were found.

The only plant character obtained in Marquillo plants examined cytologically was percentage of fruitfulness. The correlations of fruitfulness with micronuclei, nonorientation, and nonconjunction were +0.19, +0.22, and −0.06, respectively. These results are not statistically significant.

Table 9 gives the simple, partial, and multiple correlations for Marquis. Statistically none of the correlations are significant, but it is worthy of note that of the 12 simple coefficients of correlation calculated all but two are negative and these two are very low, being +0.06 and +0.10. The highest correlations were obtained between the cytologic aberrations and weight of seed, and in every case they were negative. These results indicate that there may be some correlation between cytologic aberrations and the characters of the plant in which they occur.

TABLE 9.—*Coefficients of correlation between percentage frequencies of different aberrations found occurring during microsporogenesis of Marquis and statistical measures of characters of this variety* [a]

	Simple	Partial
Number of spikes per plant (5)	$r51-0.22$	$r51.23-0.36$
	$r52-.05$	$r52.13+.29$
	$r53+.06$	$r53.12+.11$
	$R_{5.123}$ .37	
Weight of seed per plant (6)	$r61-.23$	$r61.23-.20$
	$r62-.12$	$r62.13+.06$
	$r63-.14$	$r63.12-.10$
	$R_{6.123}$ .36	
Height of plants (7)	$r71-.15$	$r71.23-.29$
	$r72+.10$	$r72.13+.29$
	$r73-.14$	$r73.12-.12$
	$R_{7.123}$ .34	
Fruitfulness of outside florets (8)	$r81-.16$	$r81.23+.03$
	$r82-.25$	$r82.13-.20$
	$r83-.04$	$r83.12-.02$
	$R_{8.123}$ .25	

[a] Key to numbers: 1=micronuclei; 2=nonorientation; 3=nonconjunction. N=24. Levels of significance: According to Fisher's (*2*) tables for the value of the correlation coefficient for different levels of significance an N of 20 and an *r* of 0.42 gives a *P* value of 0.05.

Progeny of Marquillo plants studied cytologically in 1929 were grown in 1930, and statistical measures were taken of percentage of plants emerged based upon number of seeds planted, percentage of plants matured based upon number of seeds planted, number of spikes per plant, weight of seed per plant, height of individual plants, and fruitfulness of outside florets of noncovered spikes. Coefficients of variability were calculated for these last four characters and then correlated with the percentage frequency of the different cytologic aberrations found in the parents grown in 1929.

The results of the studies on the coefficients of correlation between percentage frequencies of different aberrations found occurring during microsporogenesis of Marquillo and coefficients of variability of characters of the progeny are given in Table 10. Neither micronuclei, nonorientation, nor nonconjunction showed significant simple or partial correlations with the coefficient of variability of number of spikes per plant. Micronuclei with the coefficient of variability of weight of seed per plant gave a coefficient of correlation of $+0.51$, with nonconjunction a correlation of $+0.45$, and with nonorientation a correlation of $+0.33$. The first two are higher than $+0.42$, which is taken as the level of significance, and the latter lacks only $+0.09$ of being significant. Micronuclei and nonorientation gave significant correlations with both the coefficient of variability of height of plant and fruitfulness, being $+0.47$ and $+0.53$, respectively, in the former correlation and $+0.61$ and $+0.49$ in the latter. Nonconjunction with the coefficient of variability of these two characters gave correlations of $+0.11$ and $+0.27$, respectively. None of the partial correlations were statistically significant according to the standards set up, but all of the 12 were positive with the exception of the correlation between the coefficient of variability of number of spikes per plant and micronuclei. All of the multiple correlations were significant with the exception of number of spikes per plant correlated with micronuclei, nonorientation, and nonconjunction. These results rather definitely show that there is a positive relationship between cytologic aberrations and the coefficients of variability of the three plant characters, weight of seed, height of plants, and fruitfulness. Micronuclei and nonconjunction are more highly correlated with the coefficients of variability of weight of seed per plant than is nonorientation, and micronuclei and nonorientation are more highly correlated with the coefficients of variability of height of plants and fruitfulness than is nonconjunction. These results indicate that the micronuclei, nonorientation, and nonconjunction are causing variability in the three plant characters mentioned above or else are associated with some factor or factors causing variability.

TABLE 10.—*Coefficients of correlation between percentage frequencies of different aberrations found occurring during microsporogenesis of plants of Marquillo and coefficients of variability of characters of progeny of these plants* [a]

Characters	Simple	Partial
Number of spikes per plant (5)	$r51-0.37$	$r51.23-0.33$
	$r52-.24$	$r52.13+.02$
	$r53+.02$	$r53.12+.17$
	$R_{5\cdot123}$ .40	
Weight of seed per plant (6)	$r61+.51$	$r61.23+.32$
	$r62+.33$	$r62.13+.01$
	$r63+.45$	$r63.12+.35$
	$R_{6\cdot123}$ .60	
Height of plants (7)	$r71+.47$	$r71.23+.15$
	$r72+.53$	$r72.13+.32$
	$r73+.11$	$r73.12+.01$
	$R_{7\cdot123}$ .55	
Fruitfulness of outside florets of noncovered spikes (8)	$r81+.61$	$r81.23+.38$
	$r82+.49$	$r82.13+.13$
	$r83+.27$	$r83.12+.12$
	$R_{8\cdot123}$ .62	

[a] Key to numbers: 1=micronuclei; 2=nonorientation; 3=nonconjunction. N=23. Levels of significance: According to Fisher's (z) tables for the values of the correlation coefficient for different levels of significance an N of 20 and an r of 0.42 gives a P value of 0.05.

Table 11 gives the coefficients of correlation between percentage frequencies of different aberrations occurring during microsporogenesis of plants of Marquillo and values of statistical measurements of characters of their progeny. The aberrations used in the studies were micronuclei, nonorientation, and nonconjunction, and the characters were average number of spikes per plant, average weight of seed per plant, average percentage of fruitfulness, percentage emergence based upon number of seeds planted, and percentage matured based upon the number of seeds planted. None of the correlations with average number of spikes per plant were significant. Only four statistically significant simple correlations were obtained. Micronuclei and nonorientation gave a -0.48 and -0.42, respectively, with percentage of fruitfulness; and nonconjunction gave correlations of -0.45 and -0.46, respectively, with percentage emerged and percentage matured. The only two significant partial correlations of -0.51 and -0.50 were obtained between percentage matured and nonconjunction, holding micronuclei and nonorientation constant, and percentage matured and nonconjunction, holding the same two variables as before constant. None of the multiple correlations in which the plant character was correlated with the three chromosomal aberrations was statistically significant. None of the simple correlations between average weight of seed per plant and any of the cytologic abnormalities was statistically significant, nor were the simple correlations statistically significant between average height of plants and any of the cytologic aberrations, micronuclei, nonorientation, or nonconjunction. However, that a true correlation might exist between these two plant characters and chromosomal aberrations seems evident from the fact that the correlations obtained were negative in all cases.

TABLE 11.—*Coefficients of correlation between percentage frequencies of different aberrations found occurring during microsporogenesis of plants of Marquillo and characters of these plants* [a]

Characters	Simple	Partial
Average number spikes per plant (5)	$r51 - 0.16$	$r51.23 - 0.07$
	$r52 - .05$	$r52.13 + .04$
	$r53 - .34$	$r53.12 - .31$
	$R_{5.123} .35$	
Average weight of seed per plant (6)	$r61 - .38$	$r61.23 - .35$
	$r62 - .11$	$r62.13 + .20$
	$r63 - .34$	$r63.12 - .23$
	$R_{6.123} .48$	
Average height of plants (7)	$r71 - .19$	$r71.23 + .11$
	$r72 - .23$	$r72.13 - .24$
	$r73 - .38$	$r73.12 - .38$
	$R_{7.123} .45$	
Percentage fruitfulness of outside florets of noncovered spikes (8)	$r81 - .48$	$r81.23 - .20$
	$r82 - .42$	$r82.13 - .18$
	$r83 - .35$	$r83.12 - .26$
	$R_{8.123} .54$	
Percentage emergence based upon number of seeds planted (9)	$r91 + .02$	$r91.23 + .31$
	$r92 - .09$	$r92.13 - .25$
	$r93 - .45$	$r93.12 - .51$
	$R_{9.123} .51$	
Percentage matured based upon number of seeds planted (X)	$rX1 + .04$	$rX1.23 + .20$
	$rX2 + .05$	$rX2.13 - .06$
	$rX3 - .46$	$rX3.12 - .50$
	$R_{X.123} .50$	

[a] Key to numbers: $_1$=micronuclei; $_2$=nonorientation; $_3$=nonconjunction. N=23. Levels of significance: According to Fischer's (*2*) tables for the values of the correlation coefficient for different levels of significance an N of 20 and an r of 0.42 gives a P value of 0.05.

In summarizing it may be said that some one, or in some cases maybe all, of the cytologic abnormalities are negatively correlated with the plant characters studied except the plant character average number of spikes per plant. Micronuclei, nonorientation, and nonconjunction seem to be negatively associated with average weight of seed per plant, average height of plants, and percentage of fruitfulness; and probably nonconjunction alone is negatively associated with percentage emergence and percentage matured.

PERCENTAGE OF NATURAL CROSSING IN MARQUILLO

It is of interest to determine the amount of natural crossing in Marquillo, since Hollingshead (*7*) has found a high percentage of nonconjunction in some F_1 hybrids, especially when Garnet and Marquillo were the parents. That natural crossing in Marquillo is probably frequent has been well recognized by workers at the Minnesota Agricultural Experiment Station. Goulden and Neatby (*3*) found this to be true, and in addition they found that the amount of natural crossing varied between lines and between different years. Hayes (*5*) and Hayes and Garber (*6*) report natural crossing in wheat to vary from 2 to 3 per cent at University Farm, St. Paul, Minn.

It will be remembered from the discussion under Materials and Methods that seeds from both covered and noncovered heads were tested for susceptibility to black stem rust, form 21. Figure 1 shows the difference in susceptibility among plants. The six leaves at A are from plants grown from seed of covered spikes and show different degrees of resistance. The nine leaves at B were grown from seed of noncovered spikes, the first six on the left showing variation in degrees of resistance and the last three on the left showing susceptibility. Once they had become mixed, it would be impossible to isolate these from the susceptible leaves taken from plants of Ceres shown at C. Then, as this picture shows, it was very easy to separate the susceptible and resistant plants.

The results obtained are reported in Table 12. Plants grown from seed of covered spikes varied in percentage of susceptible plants from 0 to 1.2, depending upon the line; while the different lines grown from seed of noncovered spikes varied in susceptibility from 0 to 9.8 per cent and had an average of 3.6 ± 0.50 per cent of susceptible plants. The lines grown from seed of covered spikes showed an average of 0.4 ± 0.11 per cent of susceptible plants, and all the plants of Ceres grown as checks proved to be susceptible. The chances of the difference of 3.2 ± 0.51 per cent between covered and noncovered seed being due to the probable errors of random sampling are very small. Griffee and Hayes (*4*) have pointed out that the percentage of plants showing evidence of crossing should be doubled in order to obtain a true measure of the amount of natural crossing that takes place. On this basis, if the percentage of susceptible plants can be used as a measure of natural crossing, the amount occurring in Marquillo would be 7.2 per cent.

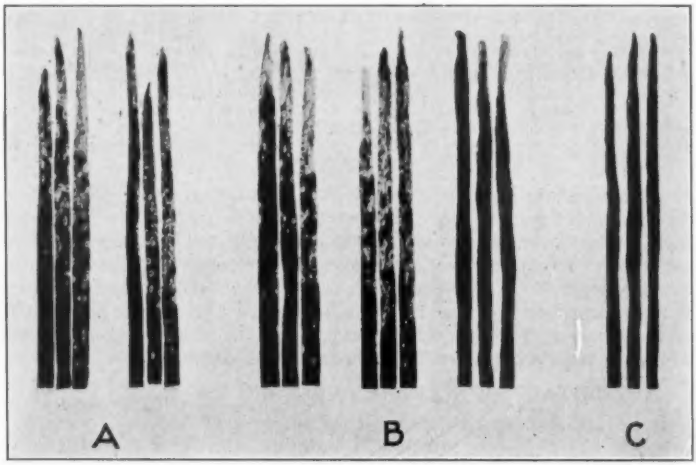

FIGURE 1.—Reaction of seedlings of Marquillo, Ceres, and natural hybrids between these two varieties to black stem rust, form 21: A, Leaves of seedlings grown from seed of covered spikes of Marquillo; B, leaves of seedlings grown from seed of noncovered spikes of Marquillo, the three at the right being natural hybrids; C, leaves of seedlings grown from seeds of Ceres

TABLE 12.—*Percentage of susceptible seedlings in different lines of Marquillo*

Culture No.	Plants	Seedlings from covered spikes			Seedlings from noncovered spikes		
		Total	Susceptible		Total	Susceptible	
	Number	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
407–12–20	19	127	0	0	214	21	9.8
407–9–3	14	114	1	0.9	169	10	5.9
407–3–417	24	243	3	1.2	371	16	4.6
313–54	21	148	0	0	245	8	3.3
407–17–12	13	104	0	0	151	4	2.6
410–55	14	164	1	.6	212	3	1.4
407–19–13	13	123	0	0	175	2	1.1
371–6	12	126	1	.8	144	0	0
Average				[a]0.4±0.11			[a]3.6±0.50

[a] Difference between those two is 3.2±0.51.

DISCUSSION

The question whether the desirable qualities of the emmer group of wheats can be combined with those of the *vulgare* group into a germinally stable variety is highly important to anyone endeavoring to improve this crop. Marquillo, Marquis, and Minnesota 2303 were used in the investigation of this problem. Marquillo was produced from a cross of Marquis with a highly rust-resistant variety of durum called Iumillo, and it combined the stem-rust resistance of the durum parents with desirable characters of the *vulgare* parent. However, under field conditions it proved to be more variable in agronomic characters than Marquis or other common wheat varieties and yielded a flour which produced bread of somewhat inferior color but satisfactory in every other particular. Marquis is grown more widely in the hard red spring wheat areas of the United States and Canada than any other variety. It is an excellent milling and baking wheat, in general possesses desirable agronomic characters, and produces fair yields except when damaged by black stem rust. Marquillo in the field is resistant to black stem rust and therefore represents a distinct step forward in combining the desirable characters of the durum and *vulgare* wheats into a single variety. Realizing the desirability of continuing beyond the progress already made, investigators at the University of Minnesota, cooperating with the Division of Cereal Crops and Diseases of the United States Department of Agriculture, crossed a sister selection of Marquillo with Kanred × Marquis selections which excelled in agronomic characters. From this cross was derived Minnesota 2303, which experiments to date have shown to be fully equal to Marquis in milling and baking qualities, including color of bread produced from the flour; superior in yielding ability; fully equal in other agronomic characters; and in addition to possess resistance to black stem rust. Minnesota 2303 combines the desirable characters of the durum and *vulgare* wheat groups. Since Marquillo exhibited more variability of agronomic characters than other common varieties, and Minnesota 2303 gives promise of becoming a highly desirable economic variety, it seemed advisable to determine their germinal stability in comparison with Marquis as evidenced by cytologic studies.

Minnesota 2303 was included in the studies on the frequency of the occurrence of micronuclei only. The results reported in this paper show that Minnesota 2303 in respect to the chromosomal aberration is no more unstable germinally than Marquis. Later unpublished work including nonorientation and nonconjunction confirmed this conclusion based upon a study of the occurrence of micronuclei. Even though Marquillo was found to be more unstable germinally than Marquis and this instability was shown to be associated with agronomic variability, the data obtained from studies with Minnesota 2303 prove that the desirable characters of the durum and common wheat groups can be combined in a germinally stable variety.

The fact that germinal instability is associated with variability of agronomic characters makes it important to determine whether this phenomenon is due to natural crossing which would produce variability and the correlations noted. This does not seem probable as the 7.2 per cent of natural crossing found in Marquillo would produce only approximately 2 hybrid plants among the 23 whose

progeny were grown for the purpose of correlating cytologic aberrations found in the parents with variability and average values of agronomic characters of the progeny. That none of these 23 plants were hybrids was indicated by the fact that it was impossible to find them segregating for any qualitative characters. Even if two hybrid lines, the number expected due to natural crossing, were present among the 23 lines studied it is evident that they could hardly have been responsible for the correlations previously reported. Moreover, it does not seem that natural crossing can explain all of the variations in percentage of chromosomal aberrations found occurring among the different plants of Marquillo and Marquis, for if natural crossing were instrumental in causing high frequencies of aberrations, these different aberrations should be positively correlated. Nonorientation did not show a significant correlation with either nonconjunction or predisjunction. Therefore, it seems that natural crossing is not responsible for the germinal instability found in Marquillo and consequently it may be concluded that germinal instability is directly responsible for at least some of the variability within agronomic characters of this variety.

These data emphasize the importance of cytology as an aid to plant breeding. More work needs to be done before the exact importance of the different chromosomal aberrations can be definitely determined. However, the data available are strongly indicative of the importance of chromosomal aberrations in producing certain phenomena pertinent to the problems which arise in any crop improvement program and consequently these phenomena should be given consideration. A statistically significant negative correlation was obtained between chromosomal aberrations and percentage fruitfulness, indicating that these aberrations may be causing sterility in some of the florets. Leighty and Taylor (*12*) and Goulden and Neatby (*3*) point out that natural crossing seems to be associated with sterility. If such is the case, it seems that chromosomal aberrations may be the indirect cause of the large percentage of natural crossing that Leighty and Taylor (*12*) reported for some varieties. Also, variations due to cytologic anomalies may be expected to behave similarly to environmental variations, and it is doubtful whether they could be eliminated by selection. For example, it was impossible to identify the 41-chromosome plants on the basis of agronomic characters.

SUMMARY

Thirty-two plants of Marquillo, 27 plants of Marquis, and 30 plants of Minnesota 2303 were studied cytologically. However, the latter variety was included only in the studies on the percentage of microspores showing micronuclei. The use of certain terms is explained. Nonorientation of bivalents has been applied to the occurrence of a bivalent or bivalents off the equatorial plane just prior to the disjunction of the main group of bivalents during metaphase of sporogenesis. (Pl. 1, C and D.) Nonconjunction is used to signify the occurrence of a univalent or univalent chromosomes during metaphase of the reductional division, when presumably the homologous mates of this univalent or these univalent chromosomes are present. (Pl. 3, A.) Polyvalence has been employed to designate the union of three or more chromosomes during the metaphase of the reductional division.

(Pls. 2, A, and 3, F.) Predisjunction is used to denote the disjunction of a bivalent or bivalents in advance of the main group of conjugated chromosomes during metaphase of the reductional division. (Pl. 3, E.)

Two of the 32 plants of Marquillo were found to have 41 somatic chromosomes, those remaining having the normal number of 42; and all of the plants of Marquis and Minnesota 2303 possessed the normal chromosome number.

Two and eight-tenths per cent of the microspores of Marquillo revealed micronuclei, while 0.8 per cent each of Marquis and Minnesota 2303 showed similar aberrations.

Extrusion of karyotin as shown in Plate 1, A, was found in 2.8 per cent of the very young microsporocytes of Marquillo, culture 407–15–17.

Nonorientation of bivalents was found to occur in 10.8 ± 0.68 per cent of the cells of the young microsporocytes of Marquillo and in 6.9 ± 0.49 per cent of those of Marquis.

Nonconjunction was exhibited in the metaphase of the first division by 6.1 ± 0.44 per cent of the Marquillo microsporocytes and in 7.7 ± 0.93 of those of Marquis.

In Marquillo on an average 1.4 per cent of the cells examined showed polyvalence, whereas 0.4 per cent of the pollen mother cells of Marquis were found to show this condition.

It does not seem that the amount of polyvalence exhibited by either Marquillo or Marquis is sufficient to account for 59.3 and 80.1 per cent, respectively, of cases of nonconjunction, which is the amount that could not be accounted for by failure of homologous chromosomes to pair.

The microsporocytes of Marquillo showed 6.3 ± 0.69 per cent of predisjunction and those of Marquis 2.8 ± 0.31 per cent.

Probably both nonorientation of bivalents and nonconjunction give rise to micronuclei found in the microspores, but it is doubtful whether predisjunction causes this phenomenon.

Microsporocytes showing fragments of chromosomes were found in both Marquillo and Marquis.

The progeny of culture 407–17–13 were grown for further cytological studies because of the high percentage of abnormalities exhibited by this plant. One of the progeny was found to possess 41 somatic chromosomes. In another progeny of this plant was found an anther in which the two pollen sacs studies exhibited differences in the percentage of microspores possessing micronuclei. In the upper pollen sac 22.3 ± 2.02 per cent of the microspores possessed micronuclei, whereas only 1.6 ± 0.64 per cent of the microspores counted in the lower pollen sac exhibited this condition.

The progeny of culture 407–12–24 were grown because the percentage of chromosomal aberrations exhibited by this plant was low. All the progeny were found to have 42 somatic chromosomes and the percentage of abnormalities was again low.

The progeny of culture 407–12–3 were grown because this plant possessed only 41 somatic chromosomes. One of the progeny was found to have 19 bivalents plus 2 univalents, whereas the other 6 possessed 41 somatic chromosomes.

An average of 23.4 ± 0.24 per cent of the microspores of the 41-chromosome plants showed micronuclei. On the basis of the num-

ber of microspores showing micronuclei the expected ratio of 20:21 chromosome gametes was found to be 74:26. Nishiyama (*14*) by using the back-cross method determined the expected ratio of 20:21 chromosome gametes to be 73:27.

The results obtained with these 42-chromosome plants strongly indicate that in somewhat more than 50 per cent of the microsporocytes in the anaphase of the first division the univalent chromosome went to the poles without dividing. The chromosomes resulting from those cases in which the univalent divided, did not divide in the second division but lagged and eventually formed the micronuclei occurring in the tetrads.

By determining the percentage of microspores showing micronuclei and making the necessary calculations, the frequency of plants having abnormal chromosome numbers may be predicted for any variety.

The coefficients of correlation calculated between percentage frequencies of different aberrations indicate that micronuclei are associated with both nonorientation of bivalents and nonconjunction, but that no correlation exists between nonorientation and nonconjunction.

The coefficients of variability of the characters of the progeny of the Marquillo plants studied cytologically were calculated and then correlated with percentage frequency of the different chromosomal aberrations. Statistically significant positive simple correlations were found between the coefficient of variability of weight of seed per plant and micronuclei as well as nonconjunction; coefficient of variability of height of plants and micronuclei as well as nonorientation of bivalents; and coefficient of variability of fruitfulness and micronuclei as well as nonorientation of bivalents.

Likewise, the average values of the characters of the progeny of the Marquillo plants studied cytologically were calculated and then correlated with percentage frequency of the different chromosomal aberrations. Statistically significant negative correlations were obtained between percentage fruitfulness and micronuclei as well as nonorientation of bivalents; between percentage emergence and nonconjunction; and between percentage of seeds which produced mature plants and nonconjunction.

The percentage of natural crossing in Marquillo was found to be 7.2, which is higher than has been generally found for other varieties at the Minnesota station.

Marquillo was found to possess greater germinal instability than Marquis, but Minnesota 2303 was found to be the equal of Marquis in germinal stability, thus proving that the desirable qualities of the durum and *vulgare* wheats can be combined into a single germinally stable variety.

The germinal instability of Marquillo was found to be associated with the greater variability of agronomic characters possessed by this variety.

The data indicate that germinal instability is responsible for the high percentage of natural crossing found in Marquillo.

Variations due to germinal instability of varieties can be distinguished from environmental variations only by combined cytologic and genetic studies.

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SOME EFFECTS OF ROOT ROT ON THE PHYSIOLOGY OF PEAS[1]

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INTRODUCTION

The disease complex known as root rot of peas (*Pisum sativum* L.) is a disturbing element in the production of the high quality in peas necessary for a Fancy canned or market product. Canners' field men have long known that in a pea field spotted with yellow-root-rot-affected areas it is difficult to determine the time to harvest the crop for maximum quality. The yellowed plants pass more rapidly up to and beyond the canning stage than the more nearly normal plants in the same field. If the field is harvested when the peas on the diseased vines reach the canning stage, a considerable yield increment that would come from the more slowly developing healthy vines is sacrificed. If, on the other hand, harvest is delayed for the yield increment from the healthy vines, the uniformity and quality of the pea pack are reduced.

Root rot occurs in scattered spots over a field of peas. The below-ground parts of affected plants turn dark, die, and rot; and the leaves become yellow and die progressively from the bottom of the plant upward. The entire diseased plant from the lowest leaf to the tip of the stem, including the pods, is a lighter shade of green than a healthy plant. Diseased plants set fewer pods and fewer peas per pod than healthy plants, and thus the yield is reduced. The symptom most significant to this study, however, is that the peas on diseased vines reach and pass the canning stage more quickly than those on healthy vines. In this way hard peas sometimes find their way into the pack.

The term "root rot" is used in this paper to denote a symptom complex. It describes most aptly the morbid condition of the pea plant when its below-ground parts are infected with pathogenic microorganisms. Several such organisms are known to occur in the soil on the canning crops farm of the New York State Agricultural Experiment Station at Geneva, N. Y., where most of the pea samples for these tests were obtained. The disease under investigation can not be ascribed with certainty to any single organism. The most important of the root-rotting fungi present are, *Pythium* spp., *Aphanomyces euteiches*, Drechs., *Fusarium martii pisi*, F. R. Jones and other species of Fusarium. *Ascochyta pinodella*, L. K. Jones, *Mycosphaerella pinodes* (Berk. and Blox.) Stone, *Rhizoctonia solani* Kühn, and species of bacteria and nematodes are also found sometimes in the roots, epicotyls, or hypocotyls of the diseased plants.

[1] Received for publication Dec. 8, 1931; issued June, 1932.

OBJECT OF THE INVESTIGATION

This paper reports a study made during the 1931 season of some of the internal chemical changes that took place in the ovules of pea plants affected with root rot. The investigation was designed primarily as a study of the aberrations in ripening and quality of canning peas that result from root rot. It is an established fact that pea root rot affects the production of canning and market garden peas, but so far as the writers are aware, this is the first attempt to evaluate the effect of root rot on the ripening and quality of peas. The significance of the maturity index of Boswell (*1*)[2] and of the quality index proposed by Sayre, Willaman, and Kertesz (*10*) was examined with reference to root-rot-affected peas.

LITERATURE REVIEW

The literature of plant pathology includes few references to the internal chemical changes that occur in plants as a result of disease. The results obtained by Shutt (*12*) and, independently, by Snyder (*13*), when reduced to a dry-matter basis, show that the grain of rusted wheat plants is higher in ash, crude fiber, and protein than the grain of healthy plants. Shutt concluded that "the growth of the rust arrests development and induces premature ripening, which * * * means a straw in which still remains the elaborated food, and a grain small, immature, rich in protein, and deficient in starch." Stoa (*14*) calculated his results on a basis of 13.5 per cent moisture, and concluded that the percentage of protein and ash decreases in the maturing grains of rusted wheat. Headden (*5*) agreed with Shutt and Snyder that the percentage of crude fiber is increased by wheat rust, but disagreed in that he found a decrease in percentage of protein.

Dungan (*3*) inoculated corn in alternate hills at planting time with various root-rotting fungi, and then analyzed the grain at harvest time. He found a decrease in specific gravity from 1.181 in the uninoculated plants to 1.154 in the inoculated. Furthermore, the inoculated plants were significantly different from the uninoculated in that they contained more nitrogen but less ether extract and total sugar. These differences were shown to be statistically significant.

The water relations of diseased plants are somewhat better known than the chemical ones. Weiss (*15*) found that the so-called water requirement of wheat plants infected with rust is higher than that of healthy plants. Kursanov (*7*) found a greater transpiration of wheat plants infected with *Ustilago tritici*, the ratio being 1:1.2. Linford (*8*) determined that peas affected with the wilt disease caused by *Fusarium orthoceras* var. *pisi* have a higher percentage of dry matter than the healthy peas. Horsfall (*6*) showed that the stunting of clover plants affected with powdery mildew was probably due to the fact that the protoplasm of the diseased plants was functioning under drier conditions than that of healthy plants. Shapovalov and Jones (*11*), working with artificially inoculated plants, confirmed field data obtained by Rosa (*9*) showing that tomato plants affected with yellows were higher in dry matter than healthy plants.

[2] Reference is made by number (italic) to Literature Cited, p. 848.

MATERIALS AND METHODS

Pea samples for this study were obtained from five fields spotted with root rot. In each field two areas of 10 to 20 square yards each were selected, one in a spot showing the well-known symptoms of root rot and the other in an adjacent spot where nearly disease-free plants were growing. A sample weighing approximately 3 kg was removed between 7 and 8 a. m. from each of these areas at various intervals, as shown in the tables. An attempt was made to get the control samples from normal-looking plants free from discoloration, although it must be stated that, as a rule, a small amount of root infection could be detected all over the field. Since the control sample consisted of disease-free or nearly disease-free plants, it is hereafter called normal.

An effort was made to trace the development of comparable peas during the canning season. Since, obviously, the same individuals could not be tested on more than one day, it was necessary to try to get comparable sections from the diseased and normal pea populations. This was done by using large numbers of vines (3 kg) and by harvesting these from contiguous areas as the season advanced.

The whole plants were always pulled, being broken at the ground line, and brought to the laboratory, where the pods were removed and shelled by hand. All samples and all determinations on the fresh peas themselves were finished within one hour after harvesting.

As soon as the sample could be shelled, it was passed through a set of hand sieves which separated it into the usual sizes 1–6, the diameters of which are given by Sayre, Willaman, and Kertesz (*10*). The total weight of peas in grams and the weight in each size were then determined. Also the total weight of vines and pods was found. From each size, where possible, quadruplicate lots of 20 unbroken peas were taken for the crushing test. The load necessary to crush each of these four lots was then determined by using Green's (*4*) modification of the crushing tester proposed by Sayre, Willaman, and Kertesz (*10*).

From these experimental data the several factors as shown in the tables were calculated. The average crushing load for the four lots of any one size was reduced to kilograms per pea. The quality index, here called QI, was calculated by adding for all sieve sizes, except size 1, the products obtained by multiplying the average crushing load of each size by the percentage of peas in that size. Boswell's number (*1*) or maturity index, here called MI, was calculated as he suggested by adding the products obtained by multiplying the percentage of peas by weight in each size by the size number. The data in the column in the tables headed "Yield of fresh peas per 100 g of vines" were obtained from the respective weights of the peas and the vines in any one sample.

For the determination of the dry matter of the peas 25 g or 50 g samples were used. These samples were dried at 100° C. for two days, after which several controls failed to show a further decrease in weight. These dried samples were used later for determining the ash, nitrogen, and crude fiber by the official methods.

PRESENTATION OF RESULTS

MATURITY AND QUALITY OF NORMAL AND ROOT-ROT-AFFECTED PEAS

ALASKA PEAS

Samples were obtained from a 1.5-acre field of Alaska peas planted on April 21, 1931, on the canning crops farm of the State experiment station.[3] The root rot occurred on the edge of the field only, in a strip 4 to 5 feet wide. On account of dry weather the peas ripened quickly but gave a good yield of Fancy grade peas. Samples were taken from the normal and from the root-rot-affected areas on June 22, 24, and 26. On June 28 one further sample was taken from the normal peas. The results obtained from this series are presented in Table 1.

TABLE 1.—*Comparison of Alaska peas from normal and from root-rot-affected vines on different dates*

Date of harvest	Normal								Root rot affected							
	Size No.	Distribution of sizes	Crushing load	QI [a]	MI [b]	Dry matter as percentage of—		Yield of fresh peas per 100 g of vines	Size No.	Distribution of sizes	Crushing load	QI [a]	MI [b]	Dry matter as percentage of—		Yield of fresh peas per 100 g of vines
						Peas	Vines							Peas	Vines	
		Per cent	*Kg per pea*					*Gms*		*Per cent*	*Kg per pea*					*Gms*
June 22	1	69.9	.....	30.4	130.1	.....	.....	.....	1	25.3	.....	122.9	203.4	.....	.....	.....
	2	30.1	1.01						2	46.0	1.45					
									3	28.7	1.96					
June 24	1	44.1	.....	93.5	181.1	.....	19.55	7.36	1	12.2	.....	192.3	258.1	.....	24.66	17.84
	2	30.7	1.41						2	30.5	1.87					
	3	25.2	1.99						3	44.3	2.32					
									4	13.0	2.50					
June 26	1	36.7	.....	125.3	202.6	17.40	21.48	14.05	1	7.8	1.85	252.3	262.4	24.25	33.12	18.68
	2	32.8	1.66			20.67			2	35.6	2.31			29.18		
	3	21.7	2.24			23.74			3	43.0	2.95			30.82		
	4	8.8	2.53			24.37			4	13.6	3.18			30.86		
June 28	1	14.7	.....	224.8	297.4	20.12	20.90	23.49	.....	.....	.....	.....	.....	.....	.....	.....
	2	17.9	1.82			24.10										
	3	30.2	2.51			25.69										
	4	29.7	3.18			32.05										
	5	7.5	3.04													

[a] Quality index. [b] Maturity index.

It is apparent that the peas increased in size very rapidly. On June 24 no peas of size 4 were found in the normal part of the field; but on June 26, 8.8 per cent and on June 28, 37 per cent of the peas harvested were size 4 or larger. While the normal peas hardened rather slowly, those affected with root rot ripened quickly. They attained a given size much earlier than the normal peas, but they did not enlarge much further. This fact is shown by the changes in the MI and QI values of these samples. In the case of the normal peas there is a steady increase in both values. In the peas from the affected area the MI value (representing enlargement) increased only from 258 to 262 after the second sample was taken. In spite of this fact the QI value increased considerably, showing the rapid hardening of the peas. There was no rainfall between June 22 and June 28. The dry-matter content of the normal plants and peas increased slowly, but that of the root-rot-affected plants and peas increased rapidly. During this interval of time there was no significant increase in the root-rot-

[3] For these samples and for the Perfection pea samples discussed later the writers are indebted to the vegetable crops division.

affected samples in yield in grams of fresh peas per 100 g of fresh vines, whereas in the normal samples the weight of fresh peas on this basis did increase.

Two other samples of Alaska peas, one from a commercial field, were obtained. These showed similar differences between normal and root-rot-affected peas. Since both of these samples were taken only on one day each, they do not by themselves give a true picture of the growth and ripening of the peas. In all these samples the root-rot-affected peas always required a higher crushing load and had a higher dry-matter content than the corresponding sample of normal peas of the same size.

Advancer Peas

The samples of Advancer peas came from a commercial field near Hall, N. Y., on June 30, July 3, and July 6. The field was harvested for Fancy peas on July 3. The results obtained are given in Table 2.

Table 2.—*Comparison of Advancer peas from normal and from root-rot-affected vines on different dates*

Date of harvest	Normal							Root rot affected						
	Size No.	Distribution of sizes	Crushing load	QI [a]	MI [b]	Dry matter as percentage of—		Size No.	Distribution of sizes	Crushing load	QI [a]	MI [b]	Dry matter as percentage of—	
						Peas	Vines						Peas	Vines
		Per cent	*Kg per pea*						*Per cent*	*Kg per pea*				
June 30	1	12.1	----	203.2	274.2	18.30	24.09	1	4.7	----	247.2	287.5	21.43	34.97
	2	17.2	1.38					2	25.2	1.77				
	3	56.3	2.45					3	48.0	2.74				
	4	13.2	3.14					4	22.1	3.23				
	5	1.2	----											
July 3	1	4.5	----	300.4	360.1	----	----	1	.7	----	317.1	378.0	----	----
	2	6.3	1.82			17.42		2	5.9	2.41			----	
	3	18.5	2.87			18.44		3	26.6	3.04			22.80	
	4	40.1	3.15			20.90		4	49.3	3.27			24.84	
	5	26.5	3.55			21.89		5	16.5	3.65			25.24	
	6	4.1	3.77			----		6	1.0	----			----	
July 6	1	1.3	----	409.9	455.9	----	----	1	1.2	----	400.7	392.5	----	----
	2	1.4	2.04			----		2	6.3	2.92			----	
	3	4.8	3.67			18.80		3	25.0	3.81			31.30	
	4	34.0	4.02			22.32		4	38.8	3.88			30.10	
	5	49.6	4.32			26.72		5	23.3	4.37			30.42	
	6	8.9	4.34			----		6	5.4	4.52			----	

[a] Quality index. [b] Maturity index.

Root rot influenced the quality of the Advancer peas in the same way as it did that of Alaska peas. Exceedingly hot weather prevailed during the period of enlargement of these peas, and no rain fell during the two weeks previous to July 2. As a result of these conditions the peas moved toward the canning stage very rapidly, as is shown by the remarkable increase in the MI value for both normal and diseased peas.

Here, again, the MI value of the normal peas continued to increase after it had become practically stationary in the root-rot-affected peas. The samples of July 6 were far below Standard grade peas. In all sizes taken on June 30 and July 3, where the two factors were determined together, the crushing loads and the dry-matter contents of the diseased samples were higher than those of the normal samples. The results obtained on the larger sizes of the samples taken on July 6 are irregular because the crushing loads obtained at that time were very near the highest limit of the machine used.

In view of the excessive dryness within the peas on the diseased vines, that contributed in part to their relative hardness, it seemed worth while to see if a part of this was not due to the withdrawal of water from the peas by the leaves. Accordingly the writers made use of the technic devised by Chandler (*2*) to demonstrate that leaves of fruit trees can extract water from the fleshy fruits. A dozen vines from each of the samples collected June 30 and July 3 were divided into two lots of six vines each, the pods being removed from six vines and left attached to six. The vines from which the pods were removed wilted approximately one hour sooner in each case than did those from which the pods were not removed, showing that leaves do have the capacity to withdraw water from the peas, thus aggravating a dry condition induced by a curtailed power of absorption through the diseased roots.

Perfection Peas

The observations on Perfection peas are the most complete obtained in this study. The peas were planted on April 15 on the canning crops farm of the experiment station. About one-third of the field was very badly injured by root rot. Because of this disease the yield obtained when the crop was harvested for Fancy peas (July 2) was much lower than the average yields previously obtained on this farm. The peas were in the Fancy grade on July 2, in the Extra Standard grade on July 3, and were Standard on July 6. The results obtained are presented in Table 3. The data that appear in Figures 1 to 8 were taken from Table 3.

TABLE 3.—*Comparison of Perfection peas from normal and from root-rot-affected vines on different dates*

Date of harvest	Normal								Root rot affected							
	Size No.	Distribution of sizes	Crushing load per pea	QI [a]	MI [b]	Dry matter as percentage of—		Yield of fresh peas per 100 g of vines	Size No.	Distribution of sizes	Crushing load per pea	QI [a]	MI [b]	Dry matter as percentage of—		Yield of fresh peas per 100 g of vines
						Peas	Vines							Peas	Vines	
		Per cent	*Kg*					*Gms*		*Per cent*	*Kg*					*Gms*
June 28	1	76.2	0.77	23.2	126.4	16.20	19.22	6.4	1	50.1	0.94	82.4	167.9	18.04	27.51	9.7
	2	21.2	.93			15.45			2	31.9	1.56			19.14		
	3	2.6	1.36						3	18.0	2.06			19.83		
July 1	1	17.0		199.3	294.3		22.98	21.4	1	7.7		243.0	301.7		27.0	19.9
	2	21.3	1.68			18.91			2	22.2	1.78			20.89		
	3	23.8	2.32			19.78			3	37.5	2.69			22.07		
	4	27.2	2.89			21.39			4	26.2	3.16			23.35		
	5	9.7	3.06			21.78			5	6.1	3.25			23.63		
	6	1.0							6	.3						
July 2	1	1.7		288.8	339.8		25.20	26.1	1	1.7		337.7	331.1		50.01	21.1
	2	18.7	1.98			18.91			2	26.9	2.95			30.79		
	3	28.6	2.88			20.14			3	19.2	3.50			29.81		
	4	40.3	3.34			21.37			4	43.9	3.72			28.85		
	5	10.5	3.30			23.34			5	7.4	3.76					
	6	.2							6	.9						
July 3	1	3.3		305.0	375.0				1	.4		346.6	391.6			
	2	4.5	1.92			18.68			2	3.2	2.73			28.70		
	3	28.9	2.97			19.80			3	26.5	3.63			27.20		
	4	41.0	3.28			20.71			4	45.4	3.44			28.21		
	5	21.8	3.49			22.11			5	23.3	3.67					
	6	.5							6	1.2						
July 6	1	.7		366.1	424.4				1	3.1		395.1	362.1			
	2	2.8	2.11						2	12.6	3.46			35.26		
	3	12.2	3.08			21.32			3	25.1	4.16			32.84		
	4	47.4	3.69			23.32			4	38.7	4.09			33.11		
	5	29.5	3.96			24.61			5	19.3	4.61					
	6	7.4	4.16						6	1.2						

[a] Quality index. [b] Maturity index.

Figure 1 shows the relation between crushing load and sieve size of peas. Each point represents the average crushing load of any given size irrespective of age. The numbers beside each point represent the average dry-matter content. It is clear from these curves, as was already well known, that the diseased peas, size for size, are harder than the normal. It seems significant, however, that the difference in crushing load between normal and diseased peas for any one size is approximately the same as that for any other size, even though the dry-matter content varies tremendously.

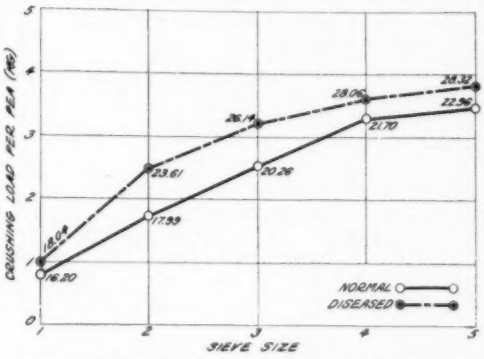

FIGURE 1.—The relation, in normal and diseased Perfection peas, between crushing load and sieve size of peas as averages for five harvest dates, showing that, size for size, the diseased peas are harder than the normal. Small figures beside the points show dry-matter content

The increase in crushing load with age of diseased and normal peas is shown in Figure 2. The points on the curves represent average crushing loads for all pea sizes weighted according to the diameter of the size in millimeters. From Figure 2 it appears that the crushing load of the normal peas increased fairly regularly with age, but the crushing load of the root-rot-affected samples was lower on July 3 than on the previous day. This can be explained only as the result of a heavy rain (0.99 inch) that fell on the evening of July 2. Sayre, Willaman, and Kertesz (*10*), working only with normal Perfection peas, found also that a rain of 0.56 inch did not reduce the load necessary to crush them. The lowered crushing load of the diseased peas after the rain would, therefore, appear significant.

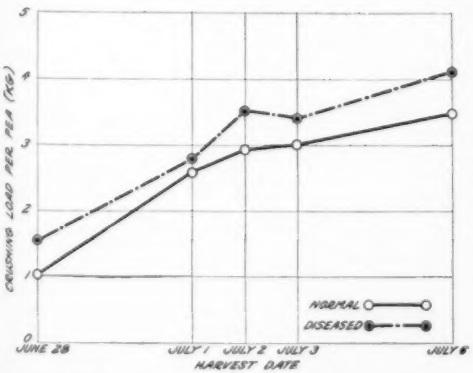

FIGURE 2.—Increase in crushing load with age, for normal and diseased Perfection peas harvested on different dates. The points on the curves represent averages for all pea sizes weighted according to the diameter of the size expressed in millimeters

The increase in dry-matter content with age of diseased and normal peas appears in Figure 3. The points on the curves represent average dry-matter content weighted, like the crushing load in Figure 2, according to the diameter of the size in millimeters. It should be noted that the drop in dry-matter content of the diseased peas after the rain of July 2 was much more pronounced than in

that of the normal peas. This drop in the percentage of dry matter for diseased peas is undoubtedly related to the decrease in the crushing load as shown in Figure 2 on the same day.

In view of the work of Sayre, Willaman, and Kertesz (*10*), who showed that crushing load (they called it crushing test) bears a close relation to dry-matter content for normal peas, it seemed worth while to plot for diseased as well as normal peas the crushing loads from Figure 2 and the dry-matter contents from Figure 3 together, using a compound vertical axis scaled on the left according to dry matter in per cent and on the right according to crushing load per pea in kilograms. This has been done in Figure 4.

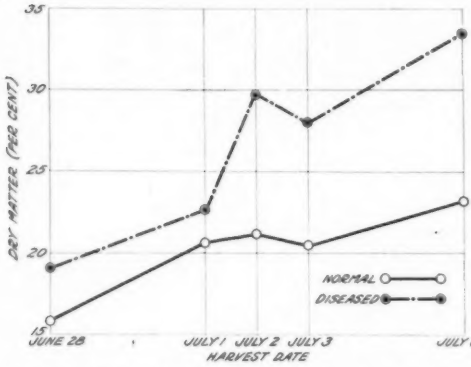

FIGURE 3.—Increase in dry-matter content with age, for normal and diseased Perfection peas harvested on different dates. The points on the curves represent averages for all pea sizes weighted like the crushing load in Figure 2 according to the diameter of the size in millimeters. It seems significant that the drop in dry-matter content of the diseased peas after the rain of July 2 should have been so much more pronounced than that of the normal peas

As was expected, the curves for dry-matter content and crushing load follow each other fairly regularly, as they should do if they bear a close relation to each other. For root-rot-affected peas, however, the dry-matter-content curve climbs relatively more rapidly with age than it should in relation to the crushing load. With this particular combination of vertical scales, the crushing-load curve for the most part remains above the dry-matter curve for the normal samples. The crushing-load curve remains altogether below the dry-matter curve for the root-rot-affected samples, however. This indicates that the composition or structure of the dry matter of the diseased peas is less resistant to crushing than that of the normal peas.

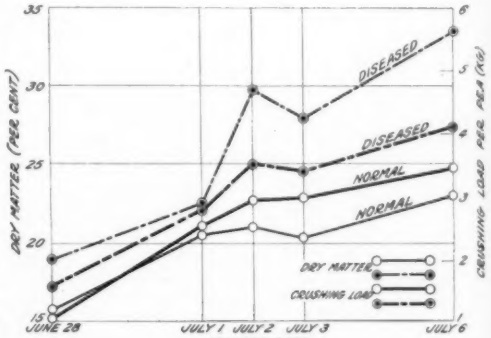

FIGURE 4.—A combination graph in which the crushing load curves from Figure 2 are combined with the dry-matter curves from Figure 3 using a compound vertical axis scaled on the left according to dry matter in per cent and on the right according to crushing load per pea in kilograms. The dry-matter curve for the diseased peas seems to climb more rapidly than it should have in relation to crushing load, as shown by the normal peas

In Figure 5 the direct relation between dry-matter content and crushing load for both diseased and normal peas are shown plotted

against each other irrespective of size or age of the samples. Reference to the curves in this figure shows, over the range in which they are comparable, that the crushing load for the peas of any particular percentage of dry matter averaged nearly 1 kg per pea lower for the root-rot-affected than for the healthy peas, thus confirming the conclusion drawn from Figure 4 that the structure or composition of the dry matter of diseased peas is less resistant to crushing than that of normal peas. Figure 5 also shows that the relation between crushing load and dry matter became much less distinct in the diseased samples when the dry matter was above 25 per cent. Unfortunately, no samples from normal peas were available to show what this relationship might have been when their dry matter was above 25 per cent.

The increase in the MI values in Table 3 is again very characteristic, showing that the increase in size of the diseased peas stopped before the normal peas attained their maximum size. The QI values of the root-rot-affected samples were always higher than those of the normal peas, on any given date. The true difference is not well expressed by these figures, however, since in the latter part of their growth the normal samples showed a higher percentage of the large sizes than did the root-rot-affected samples.

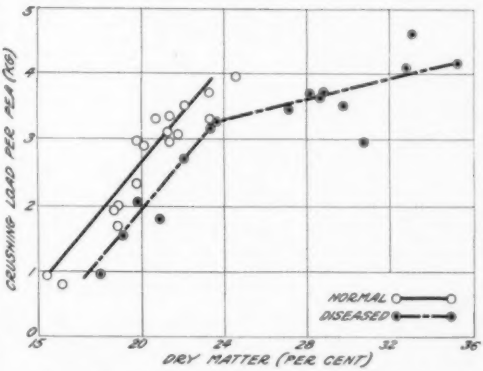

FIGURE 5.—The relation in normal and diseased Perfection peas between dry-matter content and crushing load, irrespective of sieve size or age. The diseased peas are softer than the normal on the basis of equal percentages of dry matter

The yield of fresh peas per 100 g of fresh vines was almost the same in the root-rot-affected samples taken on July 1 as in those taken on July 2, showing that the peas did not increase in weight at the expense of the vines as they did in the case of the normal samples, where this ratio increased materially. The dry-matter content of the normal vines increased between July 1 and July 2 at the usual rate, whereas, in the diseased vines, the percentage of dry matter nearly doubled, showing that the root-rot-affected vines were affected by the drought so that they dried out rapidly. Despite this decrease in the water content of the diseased plants, they did not wilt appreciably. As a result of a heavy rain (0.99 inch) that fell on the evening of July 2 the moisture deficiency of the diseased vines was somewhat relieved, as shown by the fact that the water content of the peas themselves actually increased.

On the sample of peas taken July 6, an additional test was made to determine whether pea leaves could withdraw water from the pods, thus contributing to the dryness of the peas within. This test confirmed the two reported for Advancer peas by showing that the vines without pods wilted much more quickly than those from which the pods had not been removed. Thus it is evident that the dryness of

peas on root-rot-affected plants is partly due to the removal of water from them by the leaves, which are unable to obtain from the diseased roots all the water that they require for transpiration.

CHEMICAL CHANGES IN NORMAL AND ROOT-ROT-AFFECTED PEAS

In order to obtain an insight into the changes taking place in some of the constituents of the normal and root-rot-affected peas, the percentages of ash, nitrogen, and crude fiber were determined in several of the samples. The results are presented in Tables 4, 5, and 6.

TABLE 4.—*Comparison of ash content of normal and root-rot-affected pea samples harvested on different dates*

Date of harvest	Size No.	Ash from normal peas		Ash from peas with root rot	
		Wet basis	Dry basis	Wet basis	Dry basis
		Per cent	*Per cent*	*Per cent*	*Per cent*
ALASKA VARIETY					
June 26	1	0.805	4.62	0.942	3.89
	2	.859	4.16	.940	3.22
	3	.923	3.89	.958	3.12
	4	.950	3.90	1.153	3.74
ADVANCER VARIETY					
June 26	4	0.745	3.56	0.865	3.48
	5	.755	3.45	.926	3.67
PERFECTION VARIETY					
July 1	2	0.827	4.37	0.840	4.02
	3	.884	4.47	.843	3.82
	4	.840	3.93	.869	3.72
	5	.888	4.08	.940	3.97
July 2	2	.892	4.72	1.28	4.17
	3	.819	4.07	1.23	4.12
	4	.807	3.78	1.03	3.57
	5	.901	3.86	----------	----------
July 3	2	.853	4.57	----------	----------
	3	.844	4.26	.989	3.45
	4	.880	4.10	.940	3.45
	5	.925	4.19	.948	3.36
July 6	3	.886	4.16	1.28	3.64
	4	.914	3.92	1.06	3.23
	5	.972	3.95	1.12	3.34

The percentage of ash on a fresh-weight basis was slightly higher in the root-rot-affected samples than in the controls. On a dry-matter basis, however, the percentage of ash in the diseased samples was nearly always lower, except in the Alaska sample, where it was practically the same as in the normal peas. Nitrogen also in the root-rot-affected peas was lower on a dry-matter basis than in the normal peas. No significant differences were observed in the crude-fiber content of the dry matter in the samples of Perfection peas.

TABLE 5.—*Comparison of nitrogen content of normal and root-rot-affected pea samples harvested on different dates*

ALASKA VARIETY

Date of harvest	Size No.	Nitrogen in normal peas		Nitrogen in peas with root rot	
		Wet basis	Dry basis	Wet basis	Dry basis
		Per cent	*Per cent*	*Per cent*	*Per cent*
June 26	1	0.91	5.24	0.98	4.06
	2	.99	4.77	1.02	3.51
	3	1.08	4.54	1.09	3.54
	4	1.09	4.47	1.21	3.92

ADVANCER VARIETY

June 26	4	1.02	4.87	1.07	4.29
	5	1.03	4.71	1.12	4.44

PERFECTION VARIETY

July 1	2	0.965	5.10	0.972	4.65
	3	.912	4.61	1.035	4.69
	4	.996	4.66	1.076	4.61
	5	.998	4.58	1.026	4.34
July 2	2	.940	4.97	1.336	4.34
	3	.988	4.91	1.339	4.49
	4	.982	4.59	1.296	4.49
	5	1.111	4.76		
July 3	2	1.020	5.46		
	3	.988	4.99	1.191	4.15
	4	1.030	4.98	1.195	4.40
	5	1.085	4.91	1.291	4.58
July 6	3	1.016	4.77	1.656	4.70
	4	1.101	4.72	1.451	4.42
	5	1.181	4.80	1.527	4.61

TABLE 6.—*Comparison of crude fiber content of normal and root-rot-affected Perfection pea samples harvested on different dates*

Date of harvest	Size No.	Crude fiber in normal peas		Crude fiber in peas with root rot	
		Wet basis	Dry basis	Wet basis	Dry basis
		Per cent	*Per cent*	*Per cent*	*Per cent*
July 1	2	1.36	7.26	1.54	7.38
	3	1.54	7.81	1.66	7.54
	4	1.72	8.03	1.94	7.79
	5	1.92	8.84	1.82	7.64
July 2	2	1.40	7.42	2.71	7.88
	3	1.70	8.42	2.41	8.09
	4	1.80	8.41	2.17	7.50
	5	1.95	8.36		
July 3	2	1.39	7.44		
	3	1.66	8.37	2.32	8.09
	4	2.00	9.35	2.27	8.35
	5	2.05	9.28	2.55	9.03
July 6	3	1.75	8.24	3.18	9.00
	4	1.94	8.34	3.15	9.59
	5	2.10	8.55	2.93	8.84

DISCUSSION

Both the arrested growth and the diminished quality of root-rot-affected peas seem to be associated with the low water content of the diseased plants which are living under physiologically drier conditions than the normal plants in the same field. There seems to be little doubt that the stunting of the infected plants is due to the fact that the plants are unable to supply themselves with sufficient water to carry on properly the necessary metabolic functions. In the case of the stunting of clover affected with powdery mildew (Horsfall (*6*)), the water deficiency associated with the disease probably is induced by the excessive transpiration losses through the uncuticularized hyphae of the fungus covering the leaf. In the case of root-rot-affected peas, the deficiency seems to be due to a curtailment of the power of absorption by extensive root destruction.

The decrease in water content of the root-rot-affected peas militating toward a decrease in quality, as measured by the crushing test, may come about in two ways. (1) Unlike the normals, the diseased roots are unable to supply all the water lost by transpiration, especially under drought conditions such as prevailed during the 1931 harvest season. This of itself is sufficient to increase the rate of drying in the peas and thereby lower the quality. (2) The leaves, as already shown, have the capacity of offsetting a part of their own water losses by withdrawing from the pods water that they are unable to obtain through the diseased roots, thus contributing to the hardening of the peas within.

As previously stated, the crushing load and dry-matter content of the diseased Perfection peas actually diminished after the rain on the evening of July 2, whereas these values did not diminish materially for the normal peas. (Figs. 2 and 3.) Apparently, the diseased peas absorbed enough water to replace a part of the deficiency, thus lowering the crushing load. The healthy peas, on the other hand, having no great deficiency, did not absorb enough water to lower the crushing load appreciably. The loss of water seems to be irreversible, or nearly so, in the normal samples, but not altogether irreversible in the case of the root-rot-affected samples.

In Figure 5, where the dry matter of diseased and normal peas is plotted against crushing load, irrespective of size or age of the samples, an apparently anomalous condition exists. The two factors appear to bear the same general relation to each other in both samples, as shown by the fact that the relation may be expressed by a line approximating a straight line in each case and that each line forms the same angle with the axes. Yet the curve for the diseased peas is lower for any particular percentage of dry matter by almost a kilogram per pea than for the healthy samples. A study of Figures 1 to 4 and the variation from time to time of the crushing loads of the various sizes furnished the justification for the plotting of Figure 5 without regard to size or age.

It seems significant that in both the Alaska and the Perfection samples the yield of fresh peas per 100 g of fresh vines did not increase materially beyond a certain stage in the diseased vines as it did in the normal. This indicates that the filling material either was not synthesized or did not move from the stems and leaves into the developing ovules of the root-rot-affected plants as freely as it did in the

normal plants. This condition was shown by Headden (*5*) to obtain when wheat is severely rusted. This conclusion is supported also by the fact that the MI values showed that the diseased peas failed to enlarge materially during the same period. It appears, then, that the peas began to dry out before they received their quota of filling material and were thus harder size for size than the normal peas, just as shown in Figure 1.

The question arises, why, if the diseased peas are harder size for size than the normal peas, do the curves in Figure 5 show them to be softer? Since Figure 5 was drawn to show the relation between dry matter and crushing load, the explanation must hinge on some aspect of the dry matter. An increase in percentage of dry matter in normal peas is associated with a definite increase in volume. (Fig. 6.) In the case of diseased peas, on the other hand, an increase in the percentage of dry matter is not attended by as large an increase in volume as in the normal peas. The result is that for equal percentages of dry matter a normal pea is larger and therefore harder to crush than a diseased one. For example, in Table 3 on July 1, a dry-matter content of 22.07 per cent was found in size 3 diseased peas that crushed at 2.69 kg per pea. The nearest approach to 22 per cent dry matter in the normal peas on that date was 21.78 per cent, which occurred in size 5 peas that crushed, not at 2.69, but at 3.06 kg per pea. Assuming that the constitution of the dry matter was identical in the normal and diseased peas, then the fact that the normal peas of the same percentage of dry matter are larger than diseased peas would naturally help to explain why it is harder to crush them.

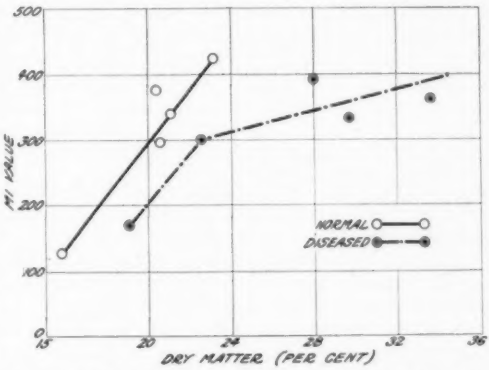

FIGURE 6.—The relation, in normal and diseased Perfection peas, between MI (maturity index) and average dry-matter content, weighted like MI according to the sieve size number of the peas. The diseased peas are smaller than normal on the basis of equal percentages of dry matter, which in part explains why they are softer

Size alone, however, is not sufficient to account for the difference in the two curves. It is illuminating in this connection to look in Table 3 not only for equal dry-matter content irrespective of size on the same day but also for equal dry-matter content of the same size irrespective of date, since neither date nor size is considered in Figure 5. The nearest approach to the 22 per cent dry-matter content for size 3 diseased peas on July 1 occurs in the normal peas on July 6 where peas containing 21.32 per cent dry matter crush, not at 2.69 kg, but at 3.08 kg per pea. Since the sizes are known to be identical, it follows that the diseased peas containing 22 per cent of dry matter were softer than the normal peas containing 21.32 per cent. Likewise size 3 diseased peas containing 19.83 per cent dry matter crushed at 2.06 kg per pea on June 28, a value matched in the

normal peas on July 1, where size 3 containing 19.78 per cent dry-matter crushed not at 2.06 but at 2.32 kg per pea. In explaining the difference on the basis of size, it was necessary to assume identical constitution of the dry matter. In explaining it on the basis of differences in structure or composition of the dry matter, assumptions are unnecessary, for the sizes are known to be identical.

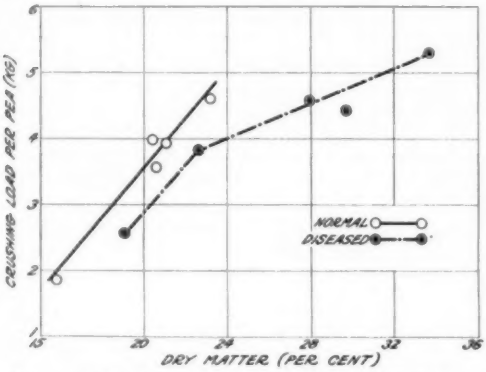

FIGURE 7.—The relation, in normal and diseased Perfection peas harvested at different dates, between dry matter and crushing load, each being weighted according to the diameter of the peas expressed in millimeters. When the effect of size is thus eliminated the diseased peas are softer than the normal on the basis of equal percentages of dry matter

The problem of separating the effect of size from that of age in the explanation of Figure 5 may be approached also mathematically by using various methods of correcting for these two factors. If such an approach be logical, then the corrected curves should show approximately the same relations between dry matter and crushing load as those in Figure 5.

Figure 7 represents an attempt to correct the crushing load and dry-matter content for size on the various harvest dates by weighting the average for any particular day according to the diameter of the pea size in millimeters. In this way all determinations for each set of samples are reduced to one for each day. Here again, it may be seen that the diseased peas are softer, dry matter for dry matter, than the normal peas.

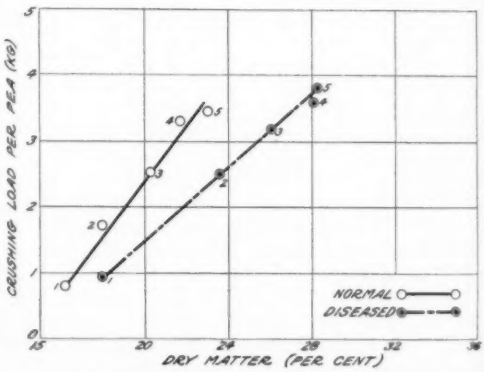

FIGURE 8.—The relation, in normal and diseased Perfection peas, between dry matter and crushing load as averages for all harvest dates. When the effect of age is thus elliminated the diseased peas are softer than the normal on the basis of equal percentages of dry matter. The small figures beside the points represent sieve size number

Figure 8 represents an attempt to correct the crushing load and dry-matter content for age by averaging the crushing loads for any particular size for all dates. The small figures adjacent to the points show pea sizes. In plotting this figure the same calculations were used as for plotting Figure 1, except that dry matter instead of size appears along the horizontal axis. Figure 8 shows again that the diseased peas

are softer than the normal peas on the basis of equal percentages of dry matter.

The maturity index as conceived by Boswell (*1*) does not fit the condition that obtains in a sample of root-rot-affected peas. Boswell's number has a significance, however, in comparing the size ratios of two samples of peas. It shows, for example, how the normal peas soon outstrip the diseased ones in enlargement. Boswell's number would rate normal peas as more nearly mature than the root-rot-affected ones of the same age that by reason of their greater dryness have more nearly reached the resting condition usually considered as maturity. It would also rate the diseased peas of the July 6 sample as less mature than those of the July 3 sample. The MI value is an admirable measure of size differences but not of maturity.

The quality index (QI) of Sayre, Willaman, and Kertesz (*10*) also fails in some measure to show the differences between the diseased and the healthy samples, because it, too, depends upon the size of the peas for its expression. This index could not be applied with success in a pea field where root rot is prevalent. Because of the actual decrease in average size for diseased Perfection peas this value is much lower on July 6 than it should have been.

SUMMARY

The effect of root rot on the physiology of peas, especially ripening and quality, was studied by following the changes in size distribution, load necessary to crush, dry matter, ash, nitrogen, and crude fiber in root-rot-affected peas as contrasted with normal peas.

According to the MI (maturity index) value, diseased peas enlarged more rapidly at first than healthy peas, but soon the rates of enlargement began to lessen, so that the diseased peas never reached the maximum size attained by normal peas.

After the growth of the diseased peas had begun to lessen their quality declined rapidly.

The curtailment of growth and the lowering of quality were both intimately associated with the lowered water content of root-rot-affected peas. This finding agrees with those of other investigators working with other plant diseases.

The load necessary to crush one pea was much higher, size for size, on the same harvest date in the diseased than in the normal samples. This is another way of saying that on the same harvest date, diseased peas were poorer in quality than normal peas. On the basis of an equal percentage of dry matter, however, the diseased peas were much softer than the normal peas. This was due to two facts: (1) They began to dry out before they were filled and thus they were smaller, hence their resistance to crushing was less; (2) the dry-matter stuff itself was softer than that in normal peas. Thus the relation between crushing load and dry matter was different in the root-rot-affected from that in the healthy peas.

No significant difference was found in the crude fiber content of normal and diseased Perfection peas.

The percentage of ash in the root-rot-affected peas on a dry-matter basis was always lower than that of the corresponding healthy peas. The percentage of nitrogen on a dry-weight basis was lower in practically every instance in diseased than in normal peas.

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THE DISTRIBUTION OF VITAMIN B COMPLEX AND ITS COMPONENTS IN THE PEANUT[1]

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INTRODUCTION

The original object of the experimentation reported in this paper was to determine the distribution of vitamin B in the various parts of the peanut kernel. At the time that the work was begun (1926) vitamin B, or water-soluble B, was quite generally considered to be a single substance, although several investigators had suggested its multiple nature. Within the next two years, however, definite proof that it contained at least two nutritive essentials had been published, and methods for their detection had been developed (*8*).[2] Consequently the scope of this investigation was enlarged to include a study of the relative quantities of the antineuritic vitamin B (B_1 or F) and of the pellagra-preventing vitamin G (B_2) present in whole raw-peanut kernels.

HISTORICAL REVIEW

Ellis and McLeod (*3, p. 343*) state that Vedder and Clark found, in 1912, that 10 g of peanuts per day protect fowls on a polished-rice diet against polyneuritis for at least 60 days. These authors also state (*3, p. 215*) that in 1918 Grieg recommended groundnut (peanut) meal biscuit as emergency rations for the Indian troops.

In 1918 Daniels and Loughlin (*2*) published evidence to show that a ration containing 75 per cent of roasted Spanish peanuts contained sufficient water-soluble B for normal growth and reproduction of rats. They also found that 56 per cent of peanut meal in the ration furnished enough of this factor for their experimental animals.

Recently Plimmer, Raymond, and Lowndes (*7*) have observed that pigeons suffered from polyneuritis when peanuts constituted 10 per cent of their ration. Twenty per cent of peanuts was enough for maintenance, and with 40 per cent rearing of young was possible. They conclude that peanuts have a relative vitamin B value of 20 when yeast is rated at 100. They say (*7, p. 546*) that this

> must at present be considered as the vitamin B_1+vitamin B_2 value, though the symptom of polyneuritis has been taken as far as possible as the criterion of the amount of vitamin in the foods.

DISTRIBUTION OF VITAMIN B COMPLEX IN THE PEANUT KERNEL

PEANUT PRODUCTS USED

Raw unshelled peanuts, together with blanched splits, hearts, and red skins from extra-large selected Virginia Runner peanuts were obtained from one of the large mills.

[1] Received for publication Nov. 18, 1931; issued June, 1932. Read before the biological section of the American Chemical Society at the Atlanta, Ga., meeting, Apr. 8, 1930.

[2] Reference is made by number (italic) to Literature Cited, p. 859.

The process of commercial blanching consists essentially in heating the graded kernels in oil at about 300° F. until the seed coats, or red skins, are loosened from the underlying tissues. The heating is not sufficient to develop a brown color in the cotyledons. The red skins and the hearts (plumules and hypocotyls) are removed mechanically from the splits or fleshy edible cotyledons.

Since each of these commercial products contained a small amount of the other parts of the nut, before being used they were further separated by hand.

Some of the shelled raw kernels were spread in thin layers in a warm room until the seed coats had dried sufficiently to be rubbed off easily. These nuts were then divided by hand into raw splits, hearts, and red skins. It was found that these kernels were composed of about 95 per cent splits, 2.3 per cent hearts, and 2.7 per cent red skins.

EXPERIMENTAL WORK

Peanut Products Incorporated in the Ration

Rats from the experiment-station stock colony, when 22 to 28 days old and weighing 35 to 50 g, were placed in cages having raised screen floors and were given the basal vitamin B free ration (Table 1) and distilled water ad libitum for 10 to 20 days, until they had ceased growing. The lot was then given a ration which contained a definite amount of one of the peanut products. One lot of rats which received 20 per cent of shelled raw peanuts was given the ration without having had the preliminary depletion period. Records were kept of the approximate daily feed intake of each lot.

Table 1.—*Composition of basal rations 195 and 196 and of supplementary ration 197 used in work on vitamin B complex*

Ingredient	Composition of ration No.—			Ingredient	Composition of ration No.—		
	195	196	197		195	196	197
	Per cent	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*
Purified casein [a]	18.0	9	....	Osborne and Mendel's salt mixture	4.0	4	16
Meat residue [b]	....	12	48	Agar-agar	2.0	2	8
Purified starch	61.0	....	....	Hydrogenated vegetable fat	15.0	9	....
Washed starch	....	58	....	Cod-liver oil	[c]0.15	6	28

[a] A nearly colorless powder free from vitamins A, B, and G, prepared according to the method given in N. C. Agr. Expt. Sta. Tech. Bul. 39 (*4, p. 122*).
[b] From a meat juice company.
[c] 0.15 g per rat per day.

The energy value of all of the experimental rations was approximately equal. These rations contained meat residue, 12 per cent; Osborne and Mendel salt mixture, 4 per cent; agar-agar, 2 per cent; and cod-liver oil, 4 per cent. The remainder consisted of the desired amount of peanut product, enough vegetable fat to make a total fat content of 25 per cent, and starch to make 100 per cent. The ration containing 50 per cent of blanched splits was an exception in that it contained 29.7 per cent of fat without the addition of a vegetable fat.

Figure 1 shows graphically the composite growth response obtained when these rations were fed to rats which had ceased growing on the

vitamin B complex free basal ration. Each composite curve is derived from the behavior of from two to six, usually three, animals in a group. All experimental rats on a given ration reacted in a rather uniform manner. The details for each individual are omitted in order to conserve space.

The average daily feed intake of the rats on these rations shows that below a critical range in the percentage of peanut products in the ration the rats do not ingest sufficient vitamin B to affect their appetite to any great extent. Within this range, however, an increase in the level of peanut product results in a much larger feed intake;

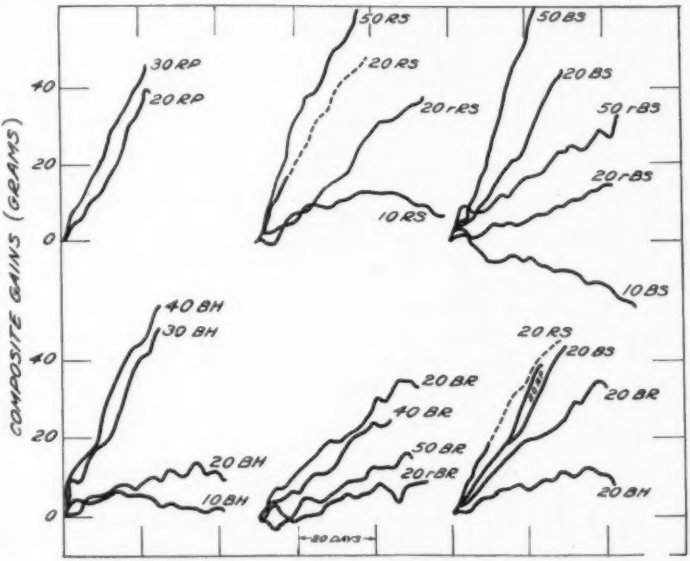

FIGURE 1.—Composite gains of rats when receiving peanut products incorporated in the ration as the sole source of vitamin B complex. The figures in the curve designations refer to the percentage of the peanut product in the ration, and the letters have the following meaning: RP, raw shelled peanuts; RS, raw splits; BS, blanched splits; BH, blanched hearts; and BR, blanched red skins. The letter r shows that the test was conducted on a product which was approximately 1 year old and had become rancid. The composition of the rations is given in the text

consequently the daily ingestion of the peanut product increases proportionately much more rapidly than the percentage in the ration.

In order to control the daily intake of the peanut product another series of experiments was run in which definite amounts of the fractions were fed daily to individual rats.

PEANUT PRODUCTS FED SEPARATELY

The same technic as that outlined above, when the peanuts were incorporated in the ration, was followed, except that the rats were separated at the end of the depletion period and kept in individual cages. They were then given daily weighed quantities of the peanut products, together with the basal ration and distilled water ad libitum.

Basal ration 195, used in the earlier part of this work, is very similar to those used for this type of work. It was found by experiment to be free from the vitamin B complex. Later, after careful preliminary experiments, basal ration 196 was substituted in order to reduce the time and expense incurred in the elaborate purification of the casein and starch. (Table 1.)

Osborne, Wakeman, and Ferry (*6*) and Sherman and MacArthur (*10*) have shown that commercial cornstarch does not contain the vitamin B complex. This observation has been confirmed in respect to the particular brand of starch used here, but as a precaution the raw starch was suspended in water and strained through a double layer of cheesecloth. After the starch had settled overnight, the water was decanted, and the starch was spread out in shallow pans and dried at room temperature. This material is designated as washed starch.

The meat residue used in ration 196 is a dry granular by-product from the commercial manufacture of beef extract. It contains approximately 80 per cent protein (N×6.25) and 10 per cent fat, and is apparently free from the antineuritic vitamin and possibly vitamin G.

In the greater part of the work rations 195 and 196 were relied upon to furnish all needed nutritive essentials for growth except the vitamin B complex. It was feared that at times the rats might not eat enough of ration 196 to supply their minimum needs, so 0.25 g of ration 197 was fed daily with the peanut product (Table 1), the basal ration being withheld until all of the supplements were eaten. This procedure insured the consumption of at least 70 mg of cod-liver oil and 40 mg of the salt mixture each day. This method of feeding was discontinued after a short time, since there was no apparent benefit from it.

Before being fed, all peanut products, except red skins, were ground in a meat chopper, care being taken to avoid crushing them to the consistency of a paste. The red skins were finely ground in a Wiley mill.

Composite gains of the animals obtained when definite quantities of the raw and blanched fractions were fed daily are shown graphically in Figure 2. For the sake of brevity the details for each rat are not given. The curves are each the composite of three to five individually fed rats which reacted rather uniformly.

It was necessary to mix the unpalatable red skins with as much of the basal ration as would be consumed in a day in order to get the rats to eat them. The other fractions of the peanut kernel were eaten greedily.

DISCUSSION OF RESULTS

Figure 1 shows that neither 10 per cent of raw splits, blanched splits, nor blanched hearts stimulated growth in the rats. When, however, the amount of raw or blanched splits was doubled (that is, increased to 20 per cent) the rats responded promptly and made good growth. Because of their improved appetite these rats ate approximately four times as much of the splits as those receiving 10 per cent in their ration.

A ration containing 20 per cent of blanched hearts was only slightly superior to the one containing 10 per cent; but when the level was increased to 30 per cent, good growth resulted.

The rats receiving 20 per cent of blanched red skins made moderate growth. Increasing the red skins to 50 per cent resulted in some-

what poorer growth even though the average daily intake of this peanut product was 2.3 g per rat. The red skins seemed to be very unpalatable. The failure of the rats to respond to the larger amounts of red skins in the ration is possibly due to the deleterious effect of the tannins or other astringents present. Cajori (*1*) has shown that the presence of tannins in pecan diets is a limiting factor for the growth of rats when pecan nuts furnished the sole source of protein in the rations.

The larger part of the work with peanut products incorporated in the ration was completed in the early part of the summer of 1927. The following spring, after the peanut products had become somewhat rancid, the work with the rations containing 20 per cent of blanched red skins, 20 per cent of blanched splits, and 20 per cent of raw splits was repeated. In each case the growth response of the rats was dis-

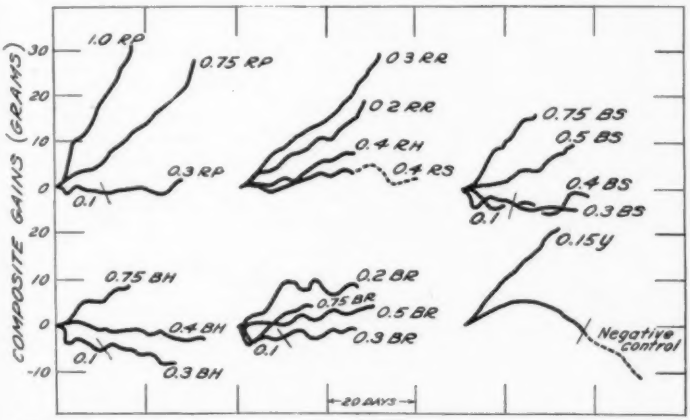

FIGURE 2.—Composite gains of rats when receiving daily weighed quantities of peanut products as the sole source of the vitamin B complex. In the curve designations the figures refer to grams of the peanut fraction fed daily, and the letters have the following meanings: RP, shelled raw peanuts; RS, raw splits; RH, raw hearts; RR, raw red skins; BS, blanched splits; BH, blanched hearts; BR, blanched red skins; and Y, dried brewers' yeast. The composition of the basal rations (Nos. 195 and 196) is given in Table 1 and discussed in the text

tinctly inferior to that obtained the preceding year (fig. 1), although the average daily feed intake was approximately the same. This indicates that the vitamin B complex was partially destroyed by storage at room temperature and that the rancidity did not affect the palatability of the rations to any marked extent.

The data (figs. 1 and 2) show that while the vitamin B complex is distributed throughout all parts of the peanut kernel, the raw red skins contain distinctly more of these factors than do the other parts. The raw hearts and raw splits apparently are about equally rich in these essentials.

Each of the blanched products contains somewhat less vitamin B complex than the corresponding raw product. This is particularly evident in the red skins but less so in the hearts and splits. The heating during the blanching process apparently destroys a large part of the thermolabile antineuritic vitamin in the thin exposed red skins but does not affect that in the body of the kernel to so great an extent.

The hearts, being small and partially exposed at the end of the nut, are affected slightly more than the splits, but much less than are the red skins.

RELATIVE QUANTITIES OF THE ANTINEURITIC AND THE PELLAGRA-PREVENTING VITAMINS IN RAW SHELLED PEANUTS

METHOD

The general method of Hunt and Krauss (*5*) was adopted as being suitable for this work, except that dried brewers' yeast which had been autoclaved at 20 pounds pressure for 4 hours was used as the source of the antipellagra vitamin. (Sherman and Axtmayer (*9*).) These investigators used the two basal rations shown in Table 2. One of these, the polyneuritic ration, 225, is devoid of both components of the vitamin B complex. The other, 223, contains the antineuritic fraction and small amounts of vitamin G. During the

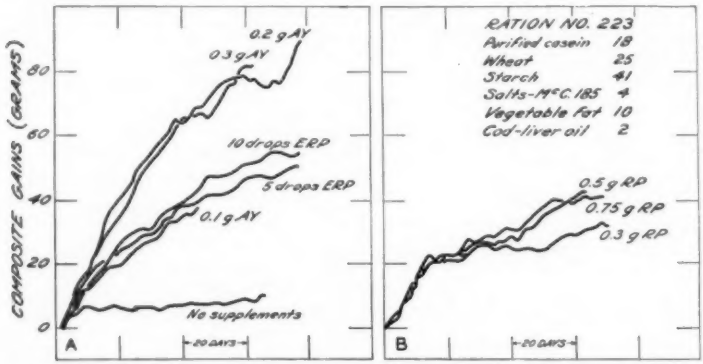

FIGURE 3.—Composite gains of rats receiving the pellagra-producing ration No. 223 without supplements, with definite quantities of autoclaved yeast (AY), or extract of rice polish (ERP) or with raw shelled peanuts (RP) fed daily: A, Controls; B, animals fed peanuts

course of this work it was found that the polyneuritic ration supplemented with an extract of rice polish was more satisfactory than the pellagra-producing ration for detecting the presence of vitamin G, because of the small but appreciable amount of this vitamin furnished by the wheat in the latter ration. The extract of rice polish was prepared in this laboratory according to the method described by Wells (*11*) for the preparation of tikitiki.[3]

TABLE 2.—*Composition of basal rations used in work on the antineuritic and pellagra-preventive vitamins*

Ingredient	Pellagra-producing ration No. 223	Polyneuritic ration No. 225	Ingredient	Pellagra-producing ration No. 223	Polyneuritic ration No. 225
	Per cent	*Per cent*		*Per cent*	*Per cent*
Purified casein	18	18	Agar-agar		2
Wheat	25		Hydrogenated vegetable fat	10	10
Washed starch	41	64	Cod-liver oil	2	2
McCollum's salts mixture 185	4	4			

[3] Tikitiki is a concentrated extract of rice polish which is prepared and distributed by the Philippine Public Health Service for the prevention of beriberi.

EXPERIMENTAL RESULTS

Controls

In all, 68 rats were used as controls and in determining the suitability of the autoclaved yeast and of the extract of rice polish as

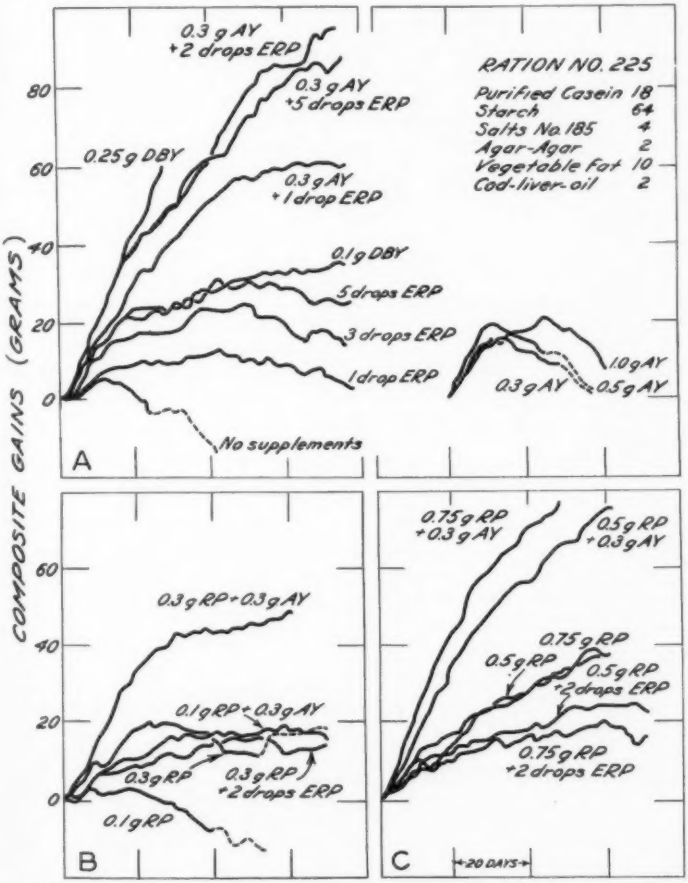

FIGURE 4.—Composite gains of control rats (A) receiving the polyneuritic ration No. 225, without supplements, or with daily definite quantities of autoclaved yeast (AY), extract of rice polish (ERP), or dried brewers' yeast (DBY); B and C, composite gains of experimental animals receiving daily ration No. 225 with definite quantities of raw shelled peanuts (RP) with and without 0.3 g autoclaved yeast and 2 drops of extract of rice polish. The dotted portion of the curves indicates that one or more of the rats had died

sources of vitamins G and B, respectively, and also the quantities that it is necessary to use for this purpose. The principal results are given in Table 3, and curves of composite gains are shown in Figures 3 and 4.

TABLE 3.—*Effect of autoclaved yeast and extract of rice polish as supplements to the pellagra-producing and the polyneuritic basal rations when fed to controls*

Rat No. and sex	Age at start	Weight at start	Quantity of supplement fed daily	Maximum weight attained	Time after start when maximum weight was attained	Duration of test period	Weight at end of test period	Average quantity of basal ration eaten daily	Average gain or loss in weight per day	Pellagra symptoms	Remarks
	Days	*Grams*		*Grams*	*Days*	*Days*	*Grams*	*Grams*	*Grams*		
PELLAGRA-PRODUCING RATION NO. 223 WITH NO SUPPLEMENT											
1973 ♂	24	46		64	64	64	64		0.28	−	
1975 ♀	24	46		54	12	64	44		−.03	−	
1981 ♂	22	48		58	9	46	46		−.04	+	
1982 ♀	22	44		60	42	46	60		.35	+	
1983 ♀	22	44		74	46	46	74		.65	−	
PELLAGRA-PRODUCING RATION NO. 223 WITH AUTOCLAVED YEAST											
2090 ♂	23	50	0.2 g	164	80	84	158	7.5	1.28	−	
2091 ♂	23	48		122	80	84	118	6.7	.83	−	
2179 ♀	22	42		130	61	63	130	7.8	1.39	−	
2024 ♀	24	44	0.5 g	118	48	48	118	7.7	1.54	−	
PELLAGRA-PRODUCING RATION NO. 223 WITH EXTRACT OF RICE POLISH											
2212 ♀	26	50	10 drops	108	78	83	108	7.0	.70	−	
2224 ♀	26	44		94	66	76	90	6.5	.61	−	
2248 ♂	26	40		102	69	76	100	6.1	.79	−	
POLYNEURITIC RATION NO. 225 WITH NO SUPPLEMENT											
2006 ♂	22	44		48	10	26	34		−.38	−	Died; spasms.
2007 ♂	22	42		48	7	26	34		−.31	−	Experiment discontinued.
2008 ♀	23	42		42	0	26	28		−.54	−	Died; spasms.
2009 ♀	23	38		40	12	26	28		−.38	−	Experiment discontinued.
2486 ♀	28	48		52	9	47	32	3.8	−.34	−	Died; spasms.
POLYNEURITIC RATION NO. 225 WITH AUTOCLAVED YEAST											
2025 ♂	24	48	0.3 g	62	10	32	38	3.6	−.31	−	Died; spasms.
2071 ♂	22	40		52	15	45	36	2.9	−.09	−	Experiment discontinued; spastic.
2072 ♂	22	42	1 g	56	15	43	36	3.3	−.14	−	Died; spasms.
2026 ♂	24	40		48	10	45	40	2.3	.00	−	Died.
2094 ♂	23	42		76	24	43	44	3.9	.05	−	Do.
2149 ♀	25	46		70	36	69	70	2.5	.35	−	Experiment discontinued.
POLYNEURITIC RATION NO. 225 WITH EXTRACT OF RICE POLISH											
2214 ♀	26	48	1 drop	62	50	83	54	4.0	.07	+	
2217 ♂	26	44		58	41	77	48	4.3	.05	+	
2250 ♀	23	42		56	34	76	40	4.3	−.03	+	
2216 ♀	26	50	5 drops	78	27	83	68	4.8	.22	+	
2219 ♀	26	44		74	41	77	64	4.9	.26	+	
2252 ♂	22	40		82	45	76	80	5.2	.53	+	
POLYNEURITIC RATION NO. 225 WITH 0.3 G AUTOCLAVED YEAST AND 2 DROPS EXTRACT OF RICE POLISH DAILY											
2525 ♂	26	42		154	90	90	154	5.6	1.24	−	
2586 ♀	25	56		150	69	96	150	5.4	.98	−	
POLYNEURITIC RATION NO. 225 WITH DRIED BREWERS' YEAST											
2031 ♂	24	52	0.25 g	110	30	33	108	6.0	1.69	−	
2032 ♂	24	52		108	33	33	108	6.6	1.69	−	
2033 ♂	24	44		110	33	33	110	6.1	2.00	−	

These control tests of the rations and of technic show that although young rats usually die within 30 days when given the polyneuritic ration only, a daily supplement of 1 drop of the extract of rice polish enables them to survive at least 80 days. However, they do not grow, and incipient symptoms of pellagra [4] develop within 50 to 70 days. When the dosage of extract of rice polish is increased to 5 or more drops slight growth results, indicating that this supplement may contain very small amounts of vitamin G, but that 2 drops daily does not furnish a sufficient quantity to be of any consequence.

The results also show that the autoclaved yeast contains only minute quantities of vitamin B. When the polyneuritic basal ration is supplemented with 2 drops of extract of rice polish and 0.3 g autoclaved yeast daily the rats are able to grow at the rate of approximately 1 g per day and maintain their health throughout the experimental period.

Rats Fed Peanuts

When tests for the presence of the antineuritic and antipellagra vitamins were made on rats fed shelled raw peanuts, the results shown in Table 4 and Figures 3 and 4 were obtained. Extra-large peanuts of the Virginia Runner variety were used. These were obtained from a different mill from the one which supplied the blanched-peanut products used in the earlier work on the vitamin B complex.

Table 4.—*Effect of shelled raw peanuts on the growth of young rats receiving the polyneuritic basal ration alone, and supplemented either with autoclaved yeast or extract of rice polish, and their effect on the growth of rats receiving the pellagra-producing basal ration*

POLYNEURITIC BASAL RATION NO. 225 WITH NO ADDITIONAL SUPPLEMENTS

Rat No. and sex	Age at start	Weight at start	Quantity of raw peanuts fed daily	Maximum weight attained	Time after start when maximum weight was attained	Duration of test period	Weight at end of test period	Average quantity of basal ration eaten daily	Average gain or loss in weight per day	Pellagra symptoms	Remarks
	Days	*Grams*	*Grams*	*Grams*	*Days*	*Days*	*Grams*	*Grams*	*Grams*		
2401 ♀	26	48	0.10	52	8	38	34	3.4	−0.37	−	Died.
2402 ♂	24	50		58	6	53	34	3.9	−.30	−	Do.
2403 ♀	26	34		36	2	69	24	2.8	−.15	−	Do.
2098 ♀	23	44	.30	64	54	83	60	4.0	.19	−	
2145 ♂	24	50		64	28	70	58	3.2	.11	−	
2146 ♀	24	46		72	53	70	64	3.3	.26	−	
2039 ♂	21	48	.50	92	60	60	92	4.3	.73	−	
2040 ♂	22	46		68	44	60	68	3.7	.37	−	
2099 ♂	23	52		104	80	83	98	4.7	.55	−	
2041 ♀	22	46	.75	68	60	60	68	4.0	.37	−	
2042 ♂	25	42		86	56	60	56	4.5	.23	−	
2100 ♂	23	50		118	83	83	118	4.8	.82	+	

[4] The term "pellagra" is used to denote vitamin G avitaminosis. It does not necessarily imply a condition identical with pellagra in man. The extreme conditions described in the literature have not been encountered in this work. The most frequent symptoms have been emaciation and ophthalmia. Sores at the corners of the mouth and considerable loss of hair have been fairly frequent. A mild dermatitis has developed occasionally, and diarrhea has been noted in a few instances.

TABLE 4.—*Effect of shelled raw peanuts on the growth of young rats receiving the polyneuritic basal ration alone, and supplemented either with autoclaved yeast or extract of rice polish, and their effect on the growth of rats receiving the pellagra-producing basal ration*—Continued

POLYNEURITIC BASAL RATION NO. 225 WITH 0.30 GRAM AUTOCLAVED YEAST PER RAT PER DAY

Rat No. and sex	Age at start	Weight at start	Quantity of raw peanuts fed daily	Maximum weight attained	Time after start when maximum weight was attained	Duration of test period	Weight at end of test period	Average quantity of basal ration eaten daily	Average gain or loss in weight per day	Pellagra symptoms	Remarks
	Days	*Grams*	*Grams*	*Grams*	*Days*	*Days*	*Grams*	*Grams*	*Grams*		
2404 ♀	26	44	0.10	64	17	52	38	3.8	−0.12	−	Died.
2405 ♀	24	46		68	27	86	64	3.9	.21	−	
2406 ♂	26	40		64	22	87	60	3.6	.23	−	
2181 ♂	22	42		86	47	60	78	4.3	.60	−	
2184 ♀	22	46	.30	110	60	62	106	4.9	.97	−	
2190 ♂	23	44		90	56	61	90	4.3	.76	−	
2182 ♀	22	38		112	49	60	112	5.4	1.23	−	
2185 ♂	22	46	.50	124	60	62	122	4.9	1.22	−	
2191 ♀	23	44		118	59	61	116	5.6	1.18	−	
2183 ♀	22	34		128	61	63	126	5.4	1.46	−	
2186 ♂	22	46	.75	146	60	62	142	5.9	1.55	−	
2192 ♀	23	36		120	56	61	116	5.6	1.31	−	

POLYNEURITIC BASAL RATION NO. 225 WITH 2 DROPS OF AN EXTRACT OF RICE POLISH • PER RAT A DAY

2410 ♂	24	46	0.30	82	69	78	74	4.4	0.36	+	
2411 ♂	24	40		52	24	78	44	3.7	.05	+	
2412 ♀	24	48		56	6	78	44	3.9	−.05	+	
2407 ♀	24	44	.50	76	83	87	80	4.8	.41	+	
2408 ♂	26	44		60	45	87	56	3.8	.14	+	
2409 ♀	26	34		64	76	87	62	3.9	.32	+	
2413 ♂	24	48	.75	66	39	78	56	3.9	.10	+	
2414 ♀	24	42		66	74	78	62	4.7	.26	−	

PELLAGRA-PRODUCING BASAL RATION NO. 223 WITH NO ADDITIONAL SUPPLEMENTS

2101 ♀	24	48	0.30	108	80	84	108	6.5	0.72	−	
2136 ♂	24	48		80	14	70	72	4.0	.34	−	
2137 ♀	24	36		78	32	70	72	4.0	.51	−	
2102 ♀	24	52	.50	124	80	84	122	7.2	.83	−	
2138 ♀	24	44		74	60	70	72	3.2	.40	−	
2139 ♂	24	48		86	63	70	84	4.4	.51	−	
2103 ♀	24	52	.75	110	77	84	110	5.5	.69	−	
2140 ♂	24	46		96	63	70	90	4.7	.63	−	
2141 ♀	24	42		74	26	70	68	3.6	.37	−	

• One drop of extract of rice polish weighed on an average of 64 mg.

DISCUSSION OF RESULTS

The results presented in Table 4 and Figure 4 show that the antineuritic vitamin contained in an extract of rice polish does not adequately supplement raw peanuts in inducing growth in young rats receiving a basal ration which is free from vitamin B complex. When, however, autoclaved yeast is substituted for the extract of rice polish the rats are able to make much greater gains in weight. It is thus seen that peanuts are relatively much richer in the antineuritic than in the pellagra-preventing fraction of vitamin B complex.

The rats made smaller gains when the peanuts were fed with the polyneuritic basal ration supplemented with an extract of rice polish

than they did when the pellagra-producing basal ration containing 25 per cent of whole wheat was used. This indicates that the wheat in ration 223 (Table 2) contained enough vitamin G to interfere with the detection of the small amount of this factor in the peanuts.

It is recognized that vitamin B complex probably contains more than the two fractions (B and G) discussed here and that more definite knowledge concerning these other factors may necessitate a modification in the methods or a reinterpretation of the results. The conclusions expressed here are justified, however, unless it shall be proven that a serious error is introduced by the absence of one or more of these little-known substances from the peanuts or the basal ration or by their presence in the autoclaved yeast or in the extract of rice polish.

SUMMARY

Commercially blanched peanut splits (cotyledons), hearts (plumules and hypocotyls), and red skins (seed coats), and the corresponding raw products from selected Virginia Runner peanuts were tested for the presence of vitamin B complex by a method which does not differentiate between the components, but in general favors the detection of the antineuritic fraction.

The raw red skins were found to contain the highest concentration of vitamin B complex, but there are appreciable quantities present in the hearts and the splits.

The process of commercial blanching (heating in oil for a short time at approximately 300° F.) destroys a large part of the vitamin present in the outer exposed red skins but does not have so marked an effect on that in the hearts and splits.

When shelled raw kernels were tested for the presence of the components of vitamin B complex it was found that they contain relatively much larger amounts of the antineuritic fraction than of the pellagra-preventing vitamin G.

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